
PHYLOGENY OF APOCYNOIDAE AND THE APSA CLADE (APOCYNACEAE S.L.)¹

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ABSTRACT

Phylogenetic relationships were reconstructed among 59 of 77 genera of subfamily Apocynoideae and exemplars of Periplocoideae, Secamonoideae, and Asclepiadoideae (collectively the APSA clade) using sequences from four regions of the chloroplast genome (*trnL* intron and *trnL-trnF* spacer, *rpl16* intron, *rps16* intron, *matK* and 3' half of *trnK* intron) and 16 morphological characters. Apocynoideae are resolved as paraphyletic. The five tribes recognized within this subfamily in the classification of Endress and Bruyns are all paraphyletic or polyphyletic. Seven major clades of Apocynoideae are identified. The first three include genera classified predominantly in tribes Wrightieae and Malouetieae sensu Endress and Bruyns and form a paraphyletic grade to a crown clade. The crown clade includes four clades of Apocynoideae genera classified in tribes Apocynae, Mesechiteae, and Echiteae together with Periplocoideae, Secamonoideae, and Asclepiadoideae; the latter three constitute the traditional Asclepiadaceae. Asclepiadaceae are resolved as polyphyletic, although the node that precludes a paraphyletic Asclepiadaceae does not have bootstrap support. The clade of Secamonoideae–Asclepiadoideae is well supported as sister to a clade of three African Apocynoideae genera (*Baissea* A. DC., *Motandra* A. DC., and *Oncinotis* Benth.). There is a strong correlation between geographic distribution and phylogeny among crown clade Apocynoideae. A New World clade is composed of American genera plus the predominantly Australasian *Parsonsia* R. Br. and *Artia* Guillaumin. An Asian clade is composed of Asian, Malesian, and Australasian genera plus the north temperate *Apocynum* L. *Trachelospermum* Lem. is polyphyletic with American and Asian species nested in the New World and Asian clades, respectively. The implications of this phylogeny for the evolution of pollen aggregation and mass transfer, the traits that were used to separate Asclepiadaceae from Apocynaceae s. str., are discussed.

Key words: Apocynaceae, Apocynoideae, APSA clade, Asclepiadaceae, biogeography, evolution, molecular phylogenetics, morphology, Periplocoideae, systematics.

APSA CLADE

The APSA clade (Table 1), subfamilies Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae of Apocynaceae s.l. (Endress & Bruyns, 2000), has been resolved as a monophyletic group in all phylogenetic analyses of the family (Judd et al., 1994; Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Potgieter & Albert, 2001).

Robert Brown (1810) divided the genera of the APSA clade between two families, Apocynaceae s. str.

(diagnosed by pollen in monads) and Asclepiadaceae (diagnosed by pollen in tetrads or pollinia; Endress, 2004). His classification was widely followed until recently (Endlicher, 1838; Schumann, 1895; Cronquist, 1981; Rosatti, 1989; Heywood, 1993).

Although never formally named under the *International Code of Botanical Nomenclature* (McNeil et al., 2006; Sennblad & Bremer [2002] coined the informal name Apocynoidina), the APSA clade was one of the first infra-familial groups recognized in Apocynaceae s.l. (Table 1). Jussieu divided Apoc-

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Table 1. Taxonomic circumscriptions. Apocynaceae s.l. includes Apocynaceae s. str. and Asclepiadaceae. Apocynaceae s. str. corresponds to subfamilies Rauvolfioideae and Apocynoideae of Apocynaceae s.l. Asclepiadaceae corresponds to subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae. The APSA clade includes the latter four subfamilies. The subfamilial classification follows Endress and Bruyns (2000).

Apocynaceae s.l.	Apocynaceae s. str.	Rauvolfioideae	APSA clade
		Apocynoideae	
	Asclepiadaceae	Periplocoideae	
		Secamonoideae	
		Asclepiadoideae	

ynaceae s.l. into three unnamed groups; one of these (diagnosed by an ovary of two free carpels, follicular fruit, and comose seeds) corresponds in circumscription to the APSA clade (Jussieu, 1789; Endress, 2004). Subsequent workers have added dextrorsely contorted corollas and lignified, basally sterile anthers (Mueller-Argoviensis, 1860), attachment of the anthers and style-head to form a gynostegium (Bentham, 1876), porate pollen (Nilsson, 1986; Nilsson et al., 1993), and the presence of steroidal alkaloids and cardenolides and absence of indole alkaloids (Hegnauer, 1988; Endress et al., 1990) to the list of diagnostic characters, making the APSA clade one of the most easily recognized groups in Apocynaceae s.l.

EVOLUTION OF COMPLEX POLLINATION MECHANISMS

Prior to the application of modern phylogenetic methods, it was already widely believed that Apocynaceae s. str. are non-monophyletic with respect to Asclepiadaceae (Table 1; Schumann, 1895; Demeter, 1922; Macfarlane, 1933). Morphological comparisons of the complex and highly modified flowers of Asclepiadaceae to the simpler flowers of Apocynaceae s. str. yielded three competing hypotheses about how the former evolved from the latter. The most widely accepted hypothesis proposes that floral morphologies of Apocynoideae, Periplocoideae, and Secamonoideae represent a succession of transitional stages between the least modified flowers of Rauvolfioideae and the most modified flowers of Asclepiadoideae (Demeter, 1922; Safwat, 1962; Cronquist, 1981; Rosatti, 1989; Endress, 1994, 2001, 2004; Endress & Bruyns, 2000; Wyatt et al., 2000). In this view, the formation of the gynostegium in Apocynoideae was followed by the evolution of increasingly efficient mechanisms of pollen aggregation and mass transfer. *Apocynum* L. (with pollen borne in tetrads and simple translators) is often regarded as the first stage in this transition

series (Demeter, 1922; Safwat, 1962; Nilsson et al., 1993), although Nilsson et al. (1993) hypothesized that tetrads in *Apocynum* and Periplocoideae are convergent, citing differences in pollen anatomy. (See discussion of translators of *Apocynum* in Appendix 2, char. 7.) Periplocoideae (with pollen in tetrads and spoon-shaped translators) have been interpreted as the next stage in the series. The tetrads are deposited onto the distal part of the spoon, which is attached to the pollinator by a sticky basal pad. Some genera of Periplocoideae have tetrads coherent into four pollinia, representing a further degree of pollen aggregation (Nilsson et al., 1993; Verhoeven & Venter, 1998). Secamonoideae have four pollinia attached to a clip-like translator (Civeyrel, 1994). They have been considered as transitional between Periplocoideae and Asclepiadoideae on the basis of several plesiomorphic features, including the presence of four sporangia per anther, predominantly non-linear microspore tetrads (Safwat, 1962), and the absence of a pollinial wall (a sporopollenin wall enclosing each pollinium; Verhoeven & Venter, 2001; Verhoeven et al., 2003). Asclepiadoideae are characterized by anthers with two rather than four sporangia and, thus, two pollinia attached to each translator of two arms (caudicles) connected to the clip-like corpusculum, linear microspore tetrads, and a pollinial wall (Frye, 1901; Safwat, 1962; Dan Dicko-Zafimahova, 1980; Verhoeven & Venter, 2001; Verhoeven et al., 2003).

An alternative view holds that Asclepiadaceae are polyphyletic with Periplocoideae nested in Rauvolfioideae and Secamonoideae–Asclepiadoideae nested in Apocynoideae (Schumann, 1895; Wanntorp, 1988). In this scenario, similarities shared by the pollination mechanisms of Periplocoideae and Secamonoideae–Asclepiadoideae, such as the presence of translators and aggregate pollen, are convergent. This hypothesis is based on a single character, the presence of unligified, fully fertile anthers (similar to those of many Rauvolfioideae) in Periplocoideae, and the frequent misinterpretation of flowers of Periplocoideae as lacking a gynostegium (Huber, 1983; Swarupandan et al., 1996). In fact, Periplocoideae have gynostegia (Nilsson et al., 1993; Endress & Bruyns, 2000) and all the other diagnostic characters of the APSA clade except lignified anthers.

Macfarlane (1933) also proposed a polyphyletic Asclepiadaceae but suggested that Periplocoideae and Secamonoideae–Asclepiadoideae are each most closely related to different genera of Apocynoideae, *Isonema* R. Br. and *Alafia* Thouars and *Baissea* A. DC. and *Oncinotis* Benth., respectively. The characters he cited to support these relationships, however, were either misinterpreted by him or are

widespread in Apocynoideae and therefore uninformative for determining relationships.

All phylogenetic studies firmly reject the idea that Periplocoideae are nested in Rauvolfioideae and instead support their inclusion in the APSA clade (Judd et al., 1994; Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Potgieter & Albert, 2001). However, only those studies with the sparsest samples of Apocynoideae have resolved a monophyletic Asclepiadaceae (Judd et al., 1994; Civeyrel et al., 1998; Lahaye et al., 2005). Studies with larger samples of Apocynoideae either do not resolve the position of Periplocoideae in the APSA clade (Potgieter & Albert, 2001) or place them as sister to various genera of Apocynoideae (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998). *Baiassea* (Apocynoideae) is resolved (but with weak support) either as sister to Secamonoideae–Asclepiadoideae (Potgieter & Albert, 2001), sister to Secamonoideae (Sennblad et al., 1998, a study that did not sample Asclepiadoideae), or intercalated between Secamonoideae and Asclepiadoideae (Sennblad & Bremer, 2002).

APOCYNODEAE

Bentham (1876) was the first to name a group that corresponds to the current circumscription of Apocynoideae as tribe Echitideae, one of three tribes of Apocynaceae s. str. Schumann (1895) first treated it as subfamily Echitoideae, a precedent that has been followed by all subsequent workers except Woodson (1930), who restricted Apocynoideae to *Apocynum* and closely related genera (distinguished by pollen in tetrads), placing all the monad-bearing genera in Echitoideae.

The inclusion of three genera, *Holarrhena* R. Br., *Spirolobium* Baill., and *Carruthersia* Seem., in Apocynoideae has been controversial. Schumann (1895) placed *Holarrhena* in Plumerioideae (Rauvolfioideae) because it lacks lignified anthers. Pichon (1950b) concurred and further removed *Spirolobium* and *Carruthersia* to Rauvolfioideae subtribe Holarrheninae because he interpreted all three genera as lacking gynostegia, although he suggested that they represent the group of Rauvolfioideae most closely related to Apocynoideae. Endress et al. (1990) showed that these three genera do have gynostegia and that they share all the other diagnostic characters of the APSA clade (except *Holarrhena* lacks lignified anthers). Leeuwenberg (1994) and Endress and Bruyns (2000) included them in Apocynoideae.

SUBDIVISION OF APOCYNODEAE

The three most widely used tribal classifications of Apocynoideae (Pichon, 1950a; Leeuwenberg, 1994;

Endress & Bruyns, 2000) are very different in their circumscriptions (Fig. 1). Of the less well-known recent systems, that of Lý (1986) is highly incongruent with each of these three, while that of Sennblad and Bremer (2002) is largely in agreement with the interpretations of Endress and Bruyns (2000).

Pichon (1948b) based his classification on a structure that is particularly difficult to observe, the region of the stamen by which it attaches to the style-head to form the gynostegium, which he termed the retinacle (Table 2). Although this classification is based on a single character, Pichon (1950a: 4) considered it phylogenetic ("the tribes composed of allied genera and probably monophyletic"; translated from the French) because, in his estimation, the state of the retinacle correlates well with the states of other characters.

Leeuwenberg (1994) criticized Pichon's tribal classification as artificial and asserted that it separated closely related genera on the basis of one difficult-to-observe character. He recognized three tribes, Wrighteae, Echiteae, and Apocyneae, dividing the genera of Mesechiteae sensu Pichon among the latter two (Fig. 1).

Endress and Bruyns (2000), like Pichon, emphasized characters of the retinacle in their classification (Table 2), although their observations were much more precise because they were based on scanning electron microscope (SEM) and serial sections (Fallen, 1986; Endress et al., 1990; Nilsson et al., 1993; Sennblad et al., 1998). They also incorporated the shape of the style-head (Table 2) and the results of several phylogenetic studies (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002). Endress and Bruyns (2000) reinstated Mesechiteae; although of the nine genera included in their circumscription, only six (three as synonyms) are among the 13 genera that constitute Mesechiteae sensu Pichon (Fig. 1). They also segregated genera from Wrighteae and Echiteae, as defined by both Leeuwenberg and Pichon, into a fifth tribe, Malouetieae (Fig. 1B).

Phylogenetic studies indicate that Apocynoideae are paraphyletic, with the genera *Wrightia* R. Br. and/or *Stephanostema* K. Schum. sister to the rest of the APSA clade (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998; Potgieter & Albert, 2001). *Holarrhena* (the only one of the three disputed genera of Pichon's subtribe Holarrheninae [Pichon, 1950b; Endress et al., 1990] included in molecular analyses to date) has been strongly supported as a member of the APSA clade (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002), corroborating its inclusion in Apocynoideae on the basis of morphological characters. In all these studies the

Table 2. Diagnostic retinacle and style-head shape characters used by Pichon (1948b, 1950a) and Endress and Bruyns (2000) as amended by Simões et al. (2004) to delimit tribes of Apocynoideae (names used by Pichon in parentheses). Pichon's descriptions are translated from the French.

	Pichon (1948b, 1950a)	Endress & Bruyns (2000)/Simões et al. (2004)	
	Retinacle	Retinacle	Style-head
Wrightiae (Nerieae)	brush of uniformly long trichomes	circular patch of trichomes	basal collar present
Malouetiae		circular patch of trichomes	basal collar absent
Echiteae (Parsonsieae)	visor of trichomes	horseshoe-shaped rim of trichomes	basal collar present
Mesechiteae (Ichnocarpeae)	glabrous facet	cellular fusion	5-ribbed
Apocyneae (Ecdysanthereae)	glabrous longitudinal ridge often accompanied by a visor of trichomes below	horseshoe-shaped rim of trichomes with a narrow longitudinal strip above	basal collar absent

relationships of most of the included genera of Apocynoideae were unresolved or only weakly supported. More recently, Simões et al. (2004) showed that Mesechiteae and Apocyneae sensu Endress and Bruyns (2000) are polyphyletic. They also showed that *Mandevilla* Lindl. is paraphyletic (Simões et al., 2006, 2007a), pointing to the need for reassessment of other generic circumscriptions.

OBJECTIVES

Much progress has been made in our understanding of the phylogeny of Apocynaceae s.l. since the analysis of Judd et al. (1994), but many questions about the APSA clade have remained unanswered. Relationships between Apocynoideae, Periplocoideae, and Secamonoideae–Asclepiadoideae are mostly unclear, precluding a test of the hypothesized transformation series leading to the highly modified flowers of Asclepiadoideae. The delimitation of genera and supergeneric groups within Apocynoideae has continued to be problematic, and most proposed circumscriptions have yet to be tested using molecular data. Because all results to date indicate that Apocynoideae are paraphyletic with respect to the other three subfamilies of the APSA clade, these two sets of problems (circumscriptions within Apocynoideae and relationships among the subfamilies) are inexorably linked. To address them, we have reconstructed phylogenetic relationships in the APSA clade using a greatly expanded sample of Apocynoideae and exemplars of the other three subfamilies.

MATERIALS AND METHODS

TAXON SAMPLING

We sampled 119 species representative of the taxonomic, morphological, and geographic diversity of

Apocynoideae. This sample comprises representatives from 59 of 77 currently recognized genera of Apocynoideae, including all seven genera of tribe Wrightiae, nine of 12 Malouetiae, 20 of 27 Apocyneae, four of six Mesechiteae, 18 of 23 Echiteae, and one of two genera incertae sedis (Appendix 1). Unless otherwise noted, the tribal and generic classification of Apocynoideae followed here is that of Endress and Bruyns (2000) as amended by Simões et al. (2004, 2006, 2007b, this issue) and Morales and Williams (2004). We sampled seven species of Periplocoideae, two with and five without pollinia, including taxa indigenous to Asia, Australia, and Africa, and representing five of the clades present in the phylogenetic analysis of the subfamily in this volume (Ionta & Judd, 2007, this issue). No well-corroborated tribal classifications are available for Secamonoideae that sample the subfamily adequately across its geographic range. Most previously published phylogenies have focused on taxa from Madagascar (Civeyrel, 1994; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2007, this issue). For this study, we chose two species of Secamonoideae, one widespread from Asia to Australia with dextrorse aestivation and without leaf colleters and the other from Asia with sinistrorse aestivation and leaf colleters. The division of Asclepiadoideae into four tribes has been well corroborated by phylogenetic analyses (e.g., Verhoeven et al., 2003); we included one species each of Fockeae and Ceropegieae, and two each of Marsdenieae and Asclepiadeae. As outgroups, we sampled 17 species from 11 genera from the tribes Carisseae, Plumerieae, and Alyxieae of Rauvolfioideae, which have been shown to be closely related to the APSA clade (Potgieter & Albert, 2001; Simões et al., 2007b). The root was placed arbitrarily at *Condyllocarpon* Desf. Multiple accessions of 11 species were sequenced for

Table 3. New amplification and sequencing primers designed for this study. Primers are numbered according to their position in our alignments and labeled F for forward and R for reverse. Polymorphic sites are indicated by standard International Union of Pure and Applied Chemistry (IUPAC) codes.

cpDNA region	Primer name	Sequence
<i>matK</i> gene/3' <i>trnK</i> intron	matK 681R	5'-GMBRAAAAYRGATTTCGTATTTCAC-3'
	matK 810F	5'-TTBATGMATTATGTKAGGTTATC-3'
	matK 1360r	5'-GTTTTHGCACAMGAAAGBCCAAG-3'
<i>rpl16</i> intron	rpl16 Fseq	5'-GCTATGCTTAGTGTGTGACTCGTTGGT-3'
	rpl16 458F	5'-GAGCTTCGRGYCAAYAAAGACTRAGAAG-3'
	rpl16 467F	5'-GYCAAYAAAGACTRAGAAGATTGACTC-3'
	rpl16 588R	5'-RCATTCAAYAGYTTHCATCGTTCCC-3'
	rpl16 643R	5'-TTYCCCCATCCCGAYCRGTG-3'
	rpl16 Rseq	5'-CATTCTTCCTCTATGTTGTTTACGG-3'
<i>rps16</i> intron	rps16 455F	5'-GGYATGTTGCTRCCATTTWGAAMCG-3'
	rps16 520R	5'-CATTGGGTTTAGRCATTMCTTCGG-3'

one or more of the sampled loci to confirm that the cladograms of chloroplast haplotypes represent species phylogeny (Shaw & Small, 2005). Voucher specimens and GenBank accession numbers are listed in Appendix 1. This is study accession number S1774 in TreeBASE.

DNA EXTRACTION

DNA was extracted with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions (except grinding was done with sand rather than liquid nitrogen) from approximately 20–30 mg of dried leaf material, either preserved in silica gel or removed from a herbarium specimen, and eluted in 160–200 µL of buffer. DNA extracts that yielded weak or no polymerase chain reaction (PCR) products were further purified and concentrated with the QIAquick PCR Purification Kit (Qiagen) using 100–200 µL of DNA extract and eluting in 30–50 µL of buffer.

PRIMERS, AMPLIFICATION, CLONING, AND SEQUENCING

We sequenced four regions of the chloroplast genome (*matK* and the 3' half of the *trnK* intron, *rpl16* intron, *rps16* intron, and *trnL* intron and *trnL-trnF* spacer) using published (*matK* 8F, 503F, 503R, 681F, 900F, 1309F, 1628R, and *trnK* 2R [Endress et al., 1996]; *rpl16* F71 and *rpl16* R1516 [Baum et al., 1998]; *rpsF* and *rpsR*2 [Oxelman et al., 1997]; *trnL* c, d, e, and *trnF* f [Taberlet et al., 1991]) and newly designed primers (Table 3).

PCR was performed in a volume of 10–25 µL containing 10 mM Tris-HCl (pH 8.6), 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTPs, 4 µg/mL BSA, 0.25 µM of each primer, 2.5 U/100 µL Taq polymerase (USB), and 0.5–1.5 µL of unquantified DNA

extract, diluted as necessary. For DNA extracts that yielded no or weak PCR products with this reaction mix, concentrations were increased to 1.6 mM dNTPs, 8 µg/mL BSA, 2 µM of each primer, and 5 U/100 µL Taq. The cycling program consisted of 4 minutes at 94 °C followed by 35 cycles of 1 minute at 94 °C, 1 minute at 55 °C, 1 or 2 (*matK*/3' *trnK*) minutes at 72 °C, and ended with 7 minutes at 72 °C, run on an MJ Research PTC-200 thermal cycler (Waltham, Massachusetts, U.S.A.).

We used primers *rps16* F and *trnK* 2R in a long PCR reaction to amplify selectively the chloroplast-encoded copy of *matK*/3' *trnK* from accessions that yielded heterogeneous sequences when amplified with primers *matK* 8F and *trnK* 2R (J. McNeal, pers. comm.). Five U/100 µL of FidelityTaq (USB) proof-reading polymerase was included in the high-concentration reaction mix described above, but with 2.75 mM MgCl₂, and cycled with the following program: 2 minutes at 94 °C, 9 cycles of 10 seconds at 94 °C, 30 seconds at 55 °C, 6 minutes at 68 °C, 15 cycles of 20 seconds at 94 °C, 30 seconds at 55 °C, 6 minutes, 20 seconds/cycle at 68 °C, 7 minutes at 68 °C (McNeal, 2005).

Unincorporated dNTPs and primers were removed either with the QIAquick PCR Purification Kit (Qiagen) or with ExoSap-IT (USB), and the purified PCR product used as the template for direct sequencing with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (v3.1; Applied Biosystems, Foster City, California, U.S.A.). Sequencing reactions were precipitated with isopropanol, resuspended in water, and analyzed on an ABI 3100 capillary sequencer (Applied Biosystems) in the Department of Organismal and Evolutionary Biology at Harvard University.

PCR products that could not be sequenced directly because of heterogeneity in lengths of simple

sequence repeats were inserted into a pGEM-T plasmid (Promega, Madison, Wisconsin, U.S.A.) and transformed into XL1-Blue competent cells (Stratagene, La Jolla, California, U.S.A.). Positive colonies were identified using blue/white screening and PCR with insert primers. Plasmids isolated with the FastPlasmid Mini Kit (Eppendorf, Hamburg, Germany) were sequenced as described above for PCR products.

SEQUENCE EDITING, ALIGNMENT, AND ANALYSIS

Sequencing traces were edited and contigs assembled with Sequencher v. 4.2 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Alignments were constructed manually using Se-Al v. 2.011a (Rambaut, 1996) and indels coded using the simple indel coding method (Simmons & Ochoterena, 2000) as implemented in GapCoder (Young & Healy, 2003). Winclada (Nixon, 2000) was used to view and manage matrices, launch tree searches in NONA (Goloboff, 1996), map characters, and view and output trees. Unalignable regions and simple sequence repeats were excluded from analysis (Table 4). Individual sequences were treated as terminals for the separate analysis of each locus. Conspecific sequences were concatenated into species terminals for the combined four-locus analysis and the combined chloroplast and morphology analysis. We implemented two searches for most parsimonious trees (MPTs) for each matrix: (1) 1000 random addition sequences, with subtree pruning and re-grafting (SPR) followed by tree bisection-reconnection (TBR) swapping, holding a maximum of 10 equally parsimonious trees per search; and (2) 10 sequential ratchet searches (Nixon, 1999), each consisting of 200 iterations, randomly reweighting 10% of the characters and randomly constraining 10% of the nodes at each iteration. Trees from the two searches were pooled and TBR-swapped until 20,000 equally parsimonious trees were saved or until the search swapped to completion. Tree length, consistency index (CI), retention index (RI), and bootstrap support (BS) were calculated on the basis of informative characters only. Bootstrap support (Felsenstein, 1985) was calculated based on 1000 pseudoreplicate matrices, each analyzed with a single random addition sequence and TBR swapping until a maximum of 20 trees were saved (Freudenstein et al., 2004). Bootstrap frequencies were calculated based on the strict consensus trees obtained from each bootstrap replicate (Davis et al., 2004). Four individual molecular data sets were analyzed (*trnL* intron/*trnL-F* spacer, *rps16* intron, *rpl16* intron, and *matK/3' trnK* intron). Congruence among these data sets was

assessed by identifying contradictory nodes with greater than 50% BS support as applied by Hufford et al. (2003).

MORPHOLOGICAL MATRIX

We reconstructed the evolution of morphological characters that are relevant to two different parts of the tree topology obtained from the molecular data set (Fig. 1, Appendices 2, 3). The first group of characters distinguishes the 18 genera of the first three branches (the *Wrightia*, *Nerium* L., and *Malouetia* A. DC. clades) from the rest of the APSA clade (the crown clade; Fig. 1). The second group of characters includes those that are the basis for the hypothesis that Periplocoideae are transitional between Apocynoideae and Secamonoideae, including proposed synapomorphies of Periplocoideae and Secamonoideae–Asclepiadoideae, as well as characters that support the monophyly of the African clade and Secamonoideae–Asclepiadoideae (Fig. 1). Whenever there are alternative interpretations of character homology, we have encoded the interpretation that favors a sister relationship between Periplocoideae and Secamonoideae–Asclepiadoideae to test if the morphological characters can overturn the resolution favored by the sequence characters (Fig. 1). All characters were constructed using reductive coding, dividing composite characters into logically and/or biologically independent ones (Wilkinson, 1995; Simmons & Freudenstein, 2002), treated as non-additive, and included in a simultaneous analysis with the combined four locus molecular data set (Nixon & Carpenter, 1996). Characters were mapped with accelerated, delayed, and unambiguous optimizations on multiple MPTs. Characters were coded based on examination of specimens held at three herbaria (A, NY, and MO), spirit-preserved material under a dissecting scope and with SEM, and serial sections by the third author, and based on literature denoted with an asterisk (*) in the Literature Cited.

RESULTS

SEQUENCES

A complete or partial sequence of each of the *trnL* intron/*trnL-F* spacer, *rps16* intron, *rpl16* intron, and *matK/3' trnK* intron was obtained for each studied species, except no *matK/3' trnK* intron sequences were obtained for three outgroup species (*Thevetia peruviana* K. Schum., *Chilocarpus rostratus* Markgr., *Pteralyxia kauaiensis* Caum) and one ingroup species (*Ichnocarpus rhombifolius* (Markgr.) D. J. Middleton), and no *rpl16* intron sequences were included for the

Table 4. Summary of analyses. For analyses of the individual loci, conspecific haplotypes are treated as separate terminals; for the combined analyses, all conspecific haplotypes are fused into species terminals. Columns that correspond to regions that are considered unalignable are included in the count of columns in the alignment. Unknown cells are quantified as percentages of the total number of cells in the matrix, i.e., the number of terminals multiplied by the number of columns in the alignment; regions with indels are not included in this count. Columns of an alignment are excluded from the analysis because they contain homopolymers or otherwise unalignable regions. Indels are coded with the simple indel coding method of Simmons and Ochoterena (2000). For each analysis, a maximum of 20,000 MPTs was saved; analyses that yielded 20,000 MPTs are presumed to have more. The count of MPTs includes ambiguously supported topologies. Length, CI, and RI are calculated based on informative characters only.

Analysis	No. of terminals	No. of columns in alignment	% cells unknown	No. of (%) alignment columns excluded from analysis	No. of informative substitutions	No. of informative indels	No. of informative morphological characters	No. of MPTs	Length of MPTs (steps)	CI of MPTs	RI of MPTs
<i>matK</i> gene/3' <i>trnK</i> intron	154	2069	2.9	34 (1.6)	629	44	0	216	2136	47	78
<i>rpl16</i> intron	156	1938	1.9	796 (41)	302	50	0	> 20,000	1126	45	77
<i>rps16</i> intron	163	1140	5.3	50 (4.4)	264	76	0	> 20,000	1048	48	76
<i>trnL</i> intron/ <i>trnL-F</i> spacer	165	1295	0.085	22 (1.7)	244	107	0	> 20,000	942	49	78
Combined molecular	150	6442	4.5	902 (14)	1414	269	0	144	5189	47	76
Combined molecular plus morphology	150	6442	4.5	902 (14)	1414	269	16	144	5250	46	76

ingroup species *Allomarkgrafia brenesiana* Woodson, *Forsteronia acouci* (Aubl.) A. DC., *F. velloziana* (A. DC.) Woodson, *Odontadenia lutea* (Vell.) Markgr., and *Rhabdadenia macrostoma* (Benth.) Müll. Arg. GenBank accession numbers are given in Appendix 1. The percentage of unknown cells in each alignment ranges from 0.085% for *trnL* intron/*trnL-F* spacer to 2.9% for *matK*/3' *trnK* intron (Table 4). A total of 4.5% of aligned cells are unknown in the concatenated matrix of all four loci (Table 4).

Heterogeneous sequences in PCR products of the *matK*/3' *trnK* intron amplified with primers *matK* 8F and *trnK* 2R were detected in *Gymnanthera oblonga* (Burm. f.) P. S. Green and *Toxocarpus villosus* (Blume) Decne. by the presence of double peaks in the sequencing traces. The same double peaks were present in sequences from at least two independent PCR reactions from two independent DNA preparations for both species. Sequences of these two species from PCR products amplified with primers *rps16* F and *trnK* 2R lacked double peaks. These were interpreted as the chloroplast-encoded copies and included in the data set.

No conspecific haplotypes (i.e., sequences from multiple accessions of a species) were resolved as polyphyletic in analyses of any of the four individual loci (results not shown). When all conspecific haplotypes were fused into species terminals for the simultaneous analysis of all four loci, 33 substitution and indel characters that are informative when individual alleles are terminals of the analysis become autapomorphic or polymorphic within species and are thus rendered uninformative, resulting in a total of 1683 informative characters for the simultaneous analysis of all four sequence partitions (Table 4).

ANALYSES

Statistical summaries of each of the six data matrices (*trnL* intron/*trnL-F* spacer, *rps16* intron, *rpl16* intron, *matK*/3' *trnK* intron, combined molecular, and combined molecular + morphology), are presented in Table 4. Alignment of three of the four molecular matrices was straightforward, with only a small percentage of positions (1.6%–4.4%) excluded because of ambiguity due to presence of homopolymers or unalignable regions (Table 4). The exception is the *rpl16* matrix, from which 41% of positions had to be excluded from analysis (Table 4). Six segments dispersed throughout the alignment were excluded, three homopolymers and three unalignable. The largest unalignable segment constituted of 626 positions of primarily imperfect repeats near the 3' end of the intron.

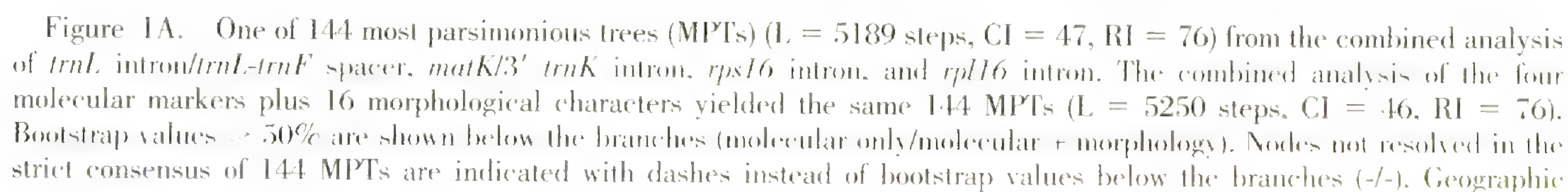
There is a single instance of contradictory clades receiving greater than 50% bootstrap support in some of the four sequence partitions and in the combined molecular analysis. In the *rps16* and *rpl16* matrices *Heterostemma* Wight & Arn. was resolved as sister to *Asclepias* L.–*Vincetoxicum* Wolfe (77% BS *rps16*, 58% BS *rpl16*), whereas in the combined molecular analysis and in the other two partitions *Heterostemma* is resolved as sister to *Marsdenia* R. Br.–*Telosma* Coville (72% BS combined, 87% BS *trnL* intron and *trnL-F* spacer, 68% BS *matK*). All other clades in each of the four sequence partitions that contradict clades in the simultaneous analysis (Fig. 1) received less than 50% bootstrap support (data not shown).

Figure 1A presents one of the 144 MPTs from the analysis of the four locus molecular data set. Addition of the sixteen morphological characters to the molecular matrix yielded the same 144 MPTs (Table 4). Eleven of the nodes in this MPT collapse in the strict consensus tree (Fig. 1, branches marked with -/-). The bootstrap values for clades in the combined morphological and molecular analysis are usually within a few percentage points of values for the molecular analysis only (Fig. 1). Unless otherwise noted, we hereafter refer to the topology and bootstrap values of the strict consensus tree for the combined molecular and morphological analysis.

PHYLOGENETIC RELATIONSHIPS, GEOGRAPHIC DISTRIBUTIONS, AND CHARACTER EVOLUTION

The APSA clade is resolved as monophyletic with 100% bootstrap support (Fig. 1A). Of its four constituent subfamilies, three, Periplocoideae, Secamonoideae, and Asclepiadoideae, are monophyletic, as is the grouping of Secamonoideae–Asclepiadoideae (all 99%–100% BS; Fig. 1B). Apocynoideae is paraphyletic. The clade comprising the three African genera, *Baissea*, *Oncinotis*, and *Motandra* A. DC. (all Apocynaceae, Apocynoideae), informally designated the African clade, is sister to Secamonoideae–Asclepiadoideae (93% BS). A crown clade including Periplocoideae, Secamonoideae, Asclepiadoideae, and all genera classified in Apocynoideae tribes Apocynaceae, Echiteae, and Mesechiteae (except *Neobraccia* Britton) is resolved with high support (97% BS). The crown clade is resolved with moderate support (81% BS) as sister to the *Malouetia* clade (70% BS). A sister relationship (100% BS) is found between the *Nerium* clade (unsupported by the bootstrap) and the crown–*Malouetia* clades. The *Wrightia* clade (100% BS) is sister to the rest of the APSA clade (100% BS).

Along with the Periplocoideae and the African clade–Secamonoideae–Asclepiadoideae, the crown



clade includes three additional subclades: a well-supported clade of Apocynoideae referred to hereafter as the Asian clade (99% BS), the genus *Rhabdadenia* Müll. Arg. (Echiteae, Apocynoideae; 100% BS), and a weakly supported clade of Apocynoideae designated the New World clade (58% BS; Fig. 1B). Relationships among these five primary subclades of the crown clade (African clade–Secamonoideae–Asclepiadoideae, Periplocoideae, *Rhabdadenia*, New World clade, and Asian clade) are not resolved in the strict consensus tree or not supported by the bootstrap (Fig. 1B).

Figure 1A–D shows the 16 morphological characters (Appendices 2, 3) mapped onto one of the MPTs with accelerated (ACCTRAN) optimization (fit: length of morphological matrix on MPTs from combined morphological and molecular matrices (L) = 61 steps, CI = 29, RI = 85). Geographic occurrence is mapped onto the same MPT as a non-additive character, and ancestral areas are reconstructed with unambiguous optimization (Fig. 1A–D).

DISCUSSION

DATA AND ANALYSIS

This study presents the best sampled, resolved, and supported phylogeny of the APSA clade to date (Fig. 1A–D). We have sampled 59 of 77 genera of Apocynoideae recognized by Endress and Bruyns (2000) as amended by Simões et al. (2004, 2006, 2007a) and Morales and Williams (2004), almost three times as many as included by Potgieter and Albert (2001) and Sennblad and Bremer (2002), who examined 22 and 21 genera of Apocynoideae, respectively. Our character matrix is more than 4.5 times larger, with 1683 informative molecular characters (Table 4), compared to 358 assembled by Potgieter and Albert (2001) and 237 by Sennblad and Bremer (2002).

The morphological matrix analyzed here is a special purpose one, composed of characters that have been used to separate taxa of the *Wrightia*, *Nerium*, and *Malouetia* clades from the crown clade, along with features that have been hypothesized as representing

a transformation series among Periplocoideae, Secamonoideae, and Asclepiadoideae. Not surprisingly, support for the *Malouetia* clade (66%–70% BS) and for the sister group relationship of the *Malouetia* and crown clades (74%–81% BS) increases when the morphological matrix is added to the combined molecular matrix (Fig. 1B), and support for the monophyly of the African clade–Secamonoideae–Asclepiadoideae decreases from 98% BS to 93% BS (Fig. 1B). The tree topology is identical with or without the morphological characters (Fig. 1); however, we consider inclusion of morphological characters in a simultaneous analysis a stronger test of our homology assessments than excluding them from the analysis and mapping them on to a molecular tree, because the latter procedure does not consider the phylogenetic evidence of the morphological characters at all and maximizes their homoplasy (Luckow & Bruneau, 1997).

The major clades resolved in this study (see Fig. 1) are discussed below with regard to their morphology and how their constituent genera were placed in the classification systems of Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000; see Table 2). Throughout this discussion, Pichon's tribes (Pichon, 1950a) will be referred to by their correct names according to the rules of priority to facilitate comparison with the other classifications.

APSA CLADE (FIG. 1A)

The APSA clade is monophyletic with 100% BS support. Attachment of the anthers and style-head to form a gynostegium, lignified anthers, porate pollen, and comose seeds have all been previously identified as synapomorphies of this clade (Judd et al., 1994; Sennblad & Bremer, 1996; Potgieter & Albert, 2001). Of the characters included in our analysis (Appendix 2), the presence of lignified anthers (char. 5, 1), secondarily lost in *Holarrhena* and Periplocoideae, and the presence of a chalazal coma (char. 10, 1) are optimized as synapomorphies for the APSA clade, although the latter is ambiguous and it is equally parsimonious to optimize this feature as evolving independently in the *Wrightia* clade and in each of

←

distributions are indicated as in the figure legend and mapped with unambiguous optimization. Morphological character changes are indicated by boxes on the branches with the character numbers above and character states below (see Appendix 2). The accelerated transformation (ACCTRAN) optimization is shown. White boxes indicate homoplastic changes, and black boxes indicate non-homoplastic changes. Ambiguously optimized character changes are indicated by an asterisk (*) next to the character number. Optimizations that are artifacts of coding inapplicable characters as unknown (Maddison, 1993) are indicated with a dagger (†) next to the character number. The position of each species of Apocynoideae in three recent classifications is indicated after the species name using the following code: P = (Pichon, 1950a), L = (Leeuwenberg, 1994), E&B/S = (Endress & Bruyns, 2000), amended by (Simões et al., 2004), (Ap) = Apocynoideae, (Ec) = Echiteae, (inc. sed.) = incertae sedis, (Ma) = Malouetieae, (Me) = Mesechiteae, (Ra) = subfamily Rauvolfioideae, (Wr) = Wrighteae.

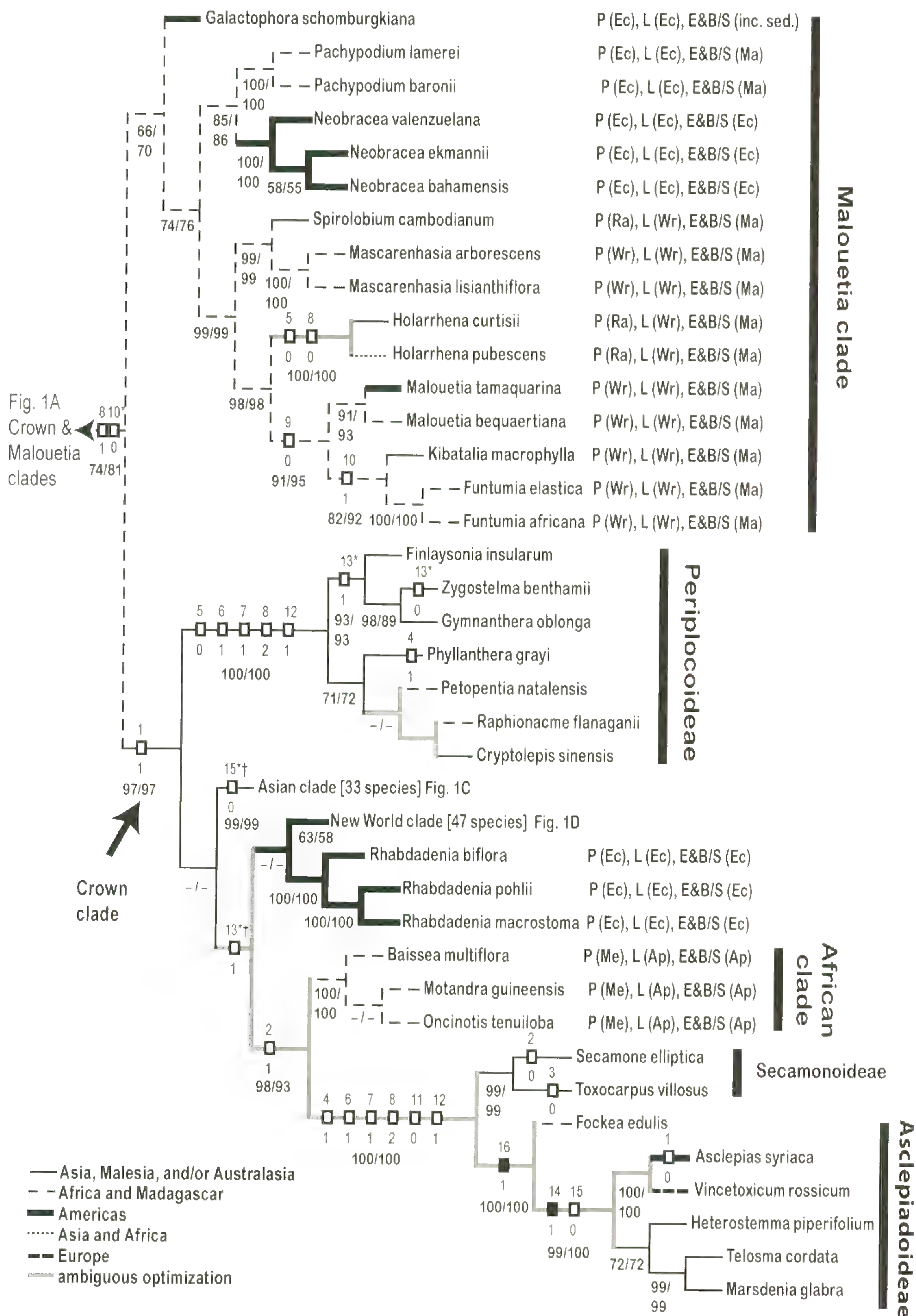


Figure 1B. Crown clade and *Malouetia* clade. See caption for Figure 1A for description.

the genera *Adenium* Roem. & Schult., *Strophanthus* DC., *Isonema*, and *Farquharia* Stapf of the *Nerium* clade (not shown). The chalazal coma of *Kibatalia* G. Don–*Funtumia* Stapf (*Malouetia* clade, Fig. 1B) is unambiguously reconstructed as a parallelism.

WRIGHTIA CLADE (FIG. 1A)

As in all previous analyses (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998; Potgieter & Albert, 2001), the *Wrightia* clade is strongly supported as

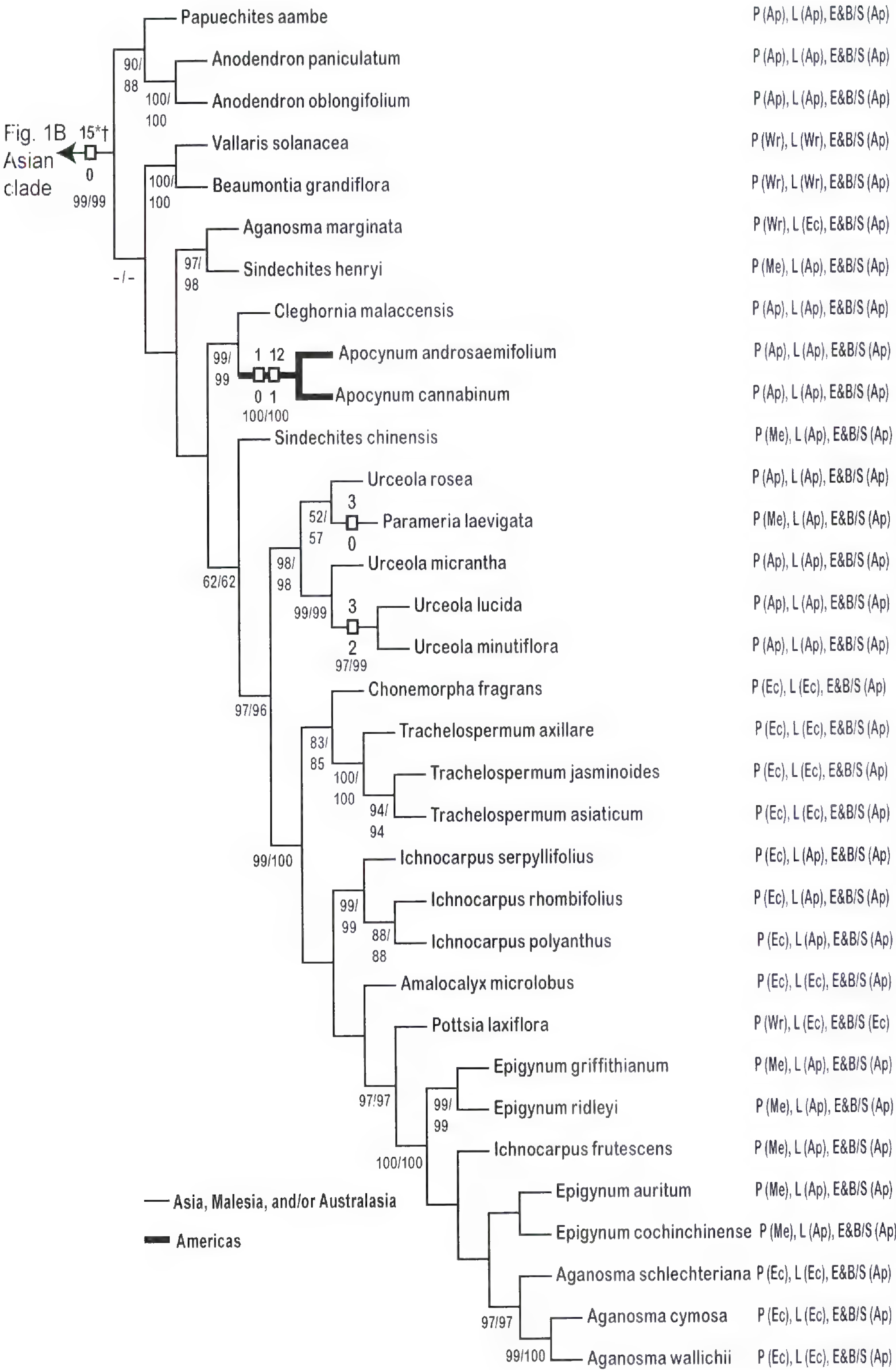


Figure 1C. Asian clade. See caption for Figure 1A for description.

monophyletic (100% BS) and as sister to the rest of the APSA clade (100% BS). The *Wrightia* clade includes three genera, two of which are endemic to Africa: *Stephanostema* (one species) and *Pleioceras* Baill. (five species; Barink, 1984), the latter analyzed here phylogenetically for the first time. The third genus, *Wrightia*, with approximately 25 species, is distributed in Africa, Asia, Malesia, Australasia, and Australia (Ngan, 1965; Forster, 1993; Middleton & Santisuk, 2001; Middleton, 2005b). The three genera have been considered as closely related (Ngan, 1965; Barink, 1984; Sennblad et al., 1998), and all three were included in tribe Wrightieae by Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000). Inclusion of the two African species of *Wrightia* would be essential to test fully its monophyly relative to the African *Stephanostema* and *Pleioceras*.

Like the analysis of Sennblad et al. (1998), the present analysis does not identify any unambiguous synapomorphies for the *Wrightia* clade. The rest of the APSA clade can be easily distinguished from this group by the presence of dextrorsely contorted corollas (char. 3, 1) and of a micropylar coma (char. 9, 1). The contrasting character states are reconstructed as plesiomorphic homologues shared by the *Wrightia* clade and Rauvolfioideae. There are two independent reversals to sinistrorse corollas in the APSA clade, one in the genus *Parameria* Benth. (Asian clade; Fig. 1C) and one in *Toxocarpus* Wight & Arn. (Secamonoideae; Fig. 1B). Secondary loss of a micropylar coma is a synapomorphy for *Malouetia*–*Kibatalia*–*Funtumia* (*Malouetia* clade; Fig. 1B).

NERIUM CLADE (FIG. 1A)

The *Nerium* clade is resolved as monophyletic in the strict consensus tree but not supported by bootstrap resampling. Previous analyses, which sampled *Nerium*, *Adenium*, and *Strophanthus* (Sennblad et al., 1998; Sennblad & Bremer, 2002) or *Nerium*, *Strophanthus*, and *Isonema* (Potgieter & Albert, 2001), have found a monophyletic *Nerium* clade with weak support (Sennblad et al., 1998) or an unresolved polytomy in the unweighted strict consensus (Potgieter & Albert, 2001; Sennblad & Bremer, 2002). Sennblad et al. (1998) identified the presence of slits in the corolla tube and elongate apical connective appendages on the anthers as synapomorphies of the *Nerium* clade, although the latter are variably present or absent in *Strophanthus* and absent from *Isonema*, *Alafia*, and *Farquharia*. The present study does not identify any synapomorphies for the *Nerium* clade, but it can be diagnosed by the combination of dextrorsely contorted corollas, presence of a micropylar coma, and absence of a nectary around the ovary (char. 8, 0).

Seeds of the genera in the *Nerium* clade either have both micropylar and chalazal comas (*Adenium*, *Strophanthus*, *Isonema*, *Farquharia*) or a micropylar coma only (*Nerium*, *Alafia*). Homology of chalazal comas in the *Nerium* clade is ambiguously optimized.

Both Pichon (1950a) and Leeuwenberg (1994) included all taxa of the *Nerium* clade in tribe Wrightieae, with the exception of *Isonema*, which Leeuwenberg transferred to Echiteae. Endress and Bruyns (2000) included all these genera in Wrightieae except *Alafia* and *Farquharia*, which they included in Malouetieae based on the structure of the style-head (Table 2). *Alafia* and *Farquharia*, however, lack the unambiguously reconstructed synapomorphy of the *Malouetia*–crown clades, a nectary around the ovary (char. 8, 1). Furthermore, *Alafia* and *Farquharia* are lianas while all other genera of Malouetieae sensu Endress and Bruyns and of the *Malouetia* clade (Fig. 1B) are exclusively trees and shrubs (with the exception of some individuals of *Galactophora schomburgkiana* Woodson).

The *Nerium* clade is divided into two well-supported subclades, *Nerium*–*Adenium* (95% BS) and *Strophanthus*–*Isonema*–*Alafia*–*Farquharia* (99% BS). *Nerium* and *Adenium* have been considered closely related to one another on the basis of similar floral morphology, particularly their extremely long, pubescent anther connectives (Pagen, 1987). Dependent growth form (char. 1, 1) is ambiguously optimized as a synapomorphy for the second subclade with a reversal to the self-supporting habit in *Strophanthus boivinii* Baill. (a Madagascan endemic that has been treated as a monotypic segregate genus, *Roupellina* (Baill.) Pichon) and with variable growth form in *Strophanthus preussii* Engl. & Pax (Beentje, 1982). An equally parsimonious reconstruction shows two independent origins of a dependent growth form in this group, one in *Strophanthus caudatus* (L.) Kurz and another in the common ancestor of *Isonema*, *Alafia*, and *Farquharia* (not shown). More extensive sampling within *Strophanthus* and a better understanding of growth forms within this genus are necessary to elucidate evolutionary patterns of this character and to test further the status of *Roupellina* and a second generic segregate of *Strophanthus*, *Christya* Ward & Harv. (not sampled here).

Alafia and the monotypic *Farquharia* have been previously suggested to be closely related (Leeuwenberg, 1997), but Zwetsloot (1981) considered the latter more closely related to *Strophanthus*. *Alafia* and *Farquharia* are resolved as a clade (56% BS), with *Farquharia* (readily distinguished by the presence of a chalazal coma [char. 10, 1] and of colleters along the petiole [char. 2, 1]) nested in a paraphyletic *Alafia*. Denser sampling of the 23 currently recognized

species of *Alafia* is necessary to further test its monophyly and to determine the status of *Farquharia*.

MALOUETIA CLADE (FIG. 1B)

Both the *Malouetia* clade (70% BS) and its sister group relationship with the crown clade (81% BS) are moderately supported. Previous analyses also resolved a *Malouetia* clade comprising *Mascarenhasia* A. DC., *Funtumia*, and *Holarrhena* (Potgieter & Albert, 2001), these three plus *Pachypodium* Lindl. (Sennblad et al., 1998), or these four genera plus *Kibatalia* (Sennblad & Bremer, 2002). The position of *Galactophora* Woodson in the *Malouetia* clade is a novel finding of the present study; it was previously part of a large polytomy in the APSA clade (Potgieter & Albert, 2001). Many of the genera of the *Malouetia* clade have been suggested to be closely related to each other on the basis of morphology, e.g., *Malouetia*, *Mascarenhasia*, *Kibatalia*, and *Funtumia* (Van der Ploeg, 1985). Sennblad et al. (1998) identified the presence of calcium oxalate packets in the anther stomium and the absence of vertical ridges on the style-head opposite the anthers as synapomorphies for this clade. While the present study does not identify any synapomorphies for the *Malouetia* clade, it is readily diagnosed by the presence of synapomorphies shared with the crown clade (presence of a nectary [char. 8, 1], secondarily lost in *Holarrhena*, and absence of a chalazal coma [char. 10, 0], regained in *Kibatalia*–*Funtumia*) and the plesiomorphic presence of the self-supporting growth form (char. 1, 0). All taxa in the *Malouetia* clade are exclusively trees or shrubs except for *Galactophora schomburgkiana*, which has both vining and shrubby varieties (Monachino, 1958; Zarucchi et al., 1995). The dependent habit is also very rare in *Galactophora*; most of the six currently recognized species are exclusively shrubs (Zarucchi et al., 1995; Morales, 2005).

All taxa of the *Malouetia* clade except *Galactophora* have cylindrical or fusiform style-heads and lack a basal collar (Table 2). The style-head of *Galactophora* has been difficult to characterize because the flowers are highly distorted by pressing. It was described and illustrated by Woodson (1936) as having five ribs and by Pichon (1950a) as having a basal collar. Simões et al. (2004) reported that it has five basal ribs distinct from the rest of the style-head. In the recent revision of the genus, the style-head is described as having five longitudinal ribs at the base, and these are clearly visible in the illustrations for each species (Morales, 2005).

Of the genera included in the *Malouetia* clade, Pichon (1950b) placed *Holarrhena* and *Spirolobium*, along with *Carruthersia* (unsampled here), in sub-

family Plumerioideae (Rauvolfioideae) as subtribe Holarrheninae of tribe Alstonieae on the basis of a misinterpretation that the flowers lacked a gynostegium (Pichon, 1950b). He placed *Mascarenhasia*, *Malouetia*, *Kibatalia*, and *Funtumia* in Wrightieae and *Galactophora*, *Pachypodium*, and *Neobraccia* in the Echiteae on the basis of their retinacle types (Table 2). The visor-like retinacle could be reconstructed as a symplesiomorphy shared by *Galactophora*, *Pachypodium*, and *Neobraccia*, with a brush retinacle evolving in the common ancestor of the rest of the *Malouetia* clade, although it would be equally parsimonious to regard the visor-like retinacle as having evolved independently in *Galactophora* and in the common ancestor of *Pachypodium* and *Neobraccia*, with the brush retinacle a symplesiomorphy shared by the rest of the *Malouetia* clade with the *Nerium* and *Wrightia* clades.

Leeuwenberg (1994) transferred *Spirolobium*, *Holarrhena*, and *Carruthersia* to Wrightieae on the basis of floral and pollen morphology and secondary chemistry as reported by Endress et al. (1990). He retained *Galactophora*, *Pachypodium*, and *Neobraccia* in Echiteae.

Endress and Bruyns (2000) classified most of the genera of the *Malouetia* clade in their tribe Malouetieae, but they retained *Neobraccia* in Echiteae, following Pichon and Leeuwenberg. They transferred *Galactophora* to Mesechiteae based on its style-head with five basal ribs (Table 2). Simões et al. (2004) found that *Galactophora* grouped with their outgroup taxa, *Pachypodium* and *Mascarenhasia*, and lacked most of the synapomorphies that they identified for Mesechiteae (see below). They treated *Galactophora* as a genus incertae sedis.

Intergeneric relationships within the *Malouetia* clade are well resolved and moderately to well supported. Loss of a micropylar coma is resolved as a synapomorphy for *Malouetia*–*Kibatalia*–*Funtumia* (char. 9, 0) and secondary gain of a chalazal coma as a synapomorphy for *Kibatalia*–*Funtumia* (char. 10, 1). Seeds of African species of *Malouetia* are pilose on both the micropylar and chalazal ends (Van der Ploeg, 1985); those of American taxa can be glabrous or pubescent, but the trichomes are not restricted to the chalazal and micropylar ends (Zarucchi et al., 1995). Our sampling of *Malouetia* is inadequate to reconstruct the evolution of seed pubescence and to judge its homology with comas in other genera. *Spirolobium* and *Holarrhena* have an unusual retinacle, inserted on the filament well below the anther (Endress et al., 1990). In the current analysis, this feature is reconstructed as a parallelism, as *Spirolobium* is sister to *Mascarenhasia* and *Holarrhena* is sister to *Malouetia*–*Kibatalia*–*Funtumia*. *Holarrhena*

has two synapomorphies, loss of lignification in the anthers (char. 5, 0) and loss of a nectary disk (char. 8, 0). This reversion to a rauvolfioid-like flower may be correlated with a change in pollinators or pollination mechanism.

CROWN CLADE (FIG. 1B)

The crown clade is strongly supported as monophyletic (97% BS). A congruent clade was resolved in several previous studies (Sennblad & Bremer, 1996, 2002; Sennblad et al., 1998). Sennblad et al. (1998) identified anthers with lignified tissue adaxial to the vascular strand and adnation of the anther thecae bases to the style-head as synapomorphies of the crown clade, but this was based on a very small sample, only seven genera of crown-clade Apocynoideae and one genus each of Secamonoideae and Periplocoideae.

The present study identifies the presence of dependent growth form (char 1, 1) as the only synapomorphy for the crown clade, although there are frequent reversals to self-supporting growth forms in taxa inhabiting more temperate and xeric habitats, e.g., *Apocynum*, *Asclepias*, *Cycladenia* Benth., *Rhodocalyx* Müll. Arg., and many species of *Mandevilla* (Fig. 1B–D), and convergent evolution of dependent growth in the *Nerium* clade (Fig. 1A), *Galactophora* (*Malouetia* clade), and in *Pleioceras* (*Wrightia* clade). Lahaye et al. (2005) identified the self-supporting growth form as plesiomorphic and the dependent growth form as apomorphic in the APSA clade, but with their much sparser sample of Apocynoideae, they could not identify at what level of the phylogeny dependent growth is apomorphic. Only Lý (1986) emphasized growth form in his tribal classification of Apocynoideae, recognizing three tribes of predominantly or entirely self-supporting genera and three of lianas, although he grouped genera with plesiomorphic and secondarily derived self-supporting growth forms together into his tribe Apocyneae.

Pichon (1950a) classified Apocynoideae genera of the crown clade in tribes Apocyneae, Echiteae, and Mesechiteae, except *Beaumontia* Wall., *Vallaris* Burm. f., *Pottsia* Hook. & Arn., and *Aganosma marginata* (Roxb.) G. Don (as part of *Amphineurion* (A. DC.) Pichon), which he included in Wrightieae. Leeuwenberg (1994) followed Pichon in his placement of *Beaumontia* and *Vallaris* but transferred *Pottsia* and *A. marginata* to Echiteae, thus classifying the vast majority of crown clade Apocynoideae in either Apocyneae or Echiteae (Fig. 1C, D). Endress and Bruyns (2000) transferred *Beaumontia* and *Vallaris* to Apocyneae, including all crown clade Apocynoideae

in their tribes Apocyneae, Echiteae, and Mesechiteae (Fig. 1C, D).

ASIAN CLADE (FIG. 1C)

The Asian clade is strongly supported as monophyletic (99% BS). All its members are endemic to tropical and subtropical Asia, Malesia, or Australasia, except *Apocynum*, which ranges across temperate Asia, southern Europe, and North America (Woodson, 1930). Significantly, all Asian species of *Trachelospermum* Lem. are part of the Asian clade, whereas the American species, *T. difforme* (Walter) A. Gray, is part of the New World clade (Fig. 1D, see discussion below). In previous phylogenetic studies, only Potgieter and Albert (2001) and Simões et al. (2004) resolved a monophyletic Asian clade. The present study does not identify any synapomorphies for the Asian clade.

Pichon (1950a) scattered taxa of the Asian clade among all four of his tribes, and Leeuwenberg (1994) likewise dispersed genera of the Asian clade among all three of the tribes he recognized.

Endress and Bruyns (2000) included all genera of the Asian clade in Apocyneae except *Pottsia*, which they left in Echiteae. This reflects the incorporation of the results of molecular analyses that supported the monophyly of morphologically divergent taxa. They also incorporated additional morphological characters, such as a very short stamen–corolla tube and a fusiform style-head without a basal collar (Endress & Bruyns, 2000; Table 2). The Asian clade is polymorphic for both of these characters, however. Relatively elongate stamen–corolla tubes (i.e., the congenitally fused part of the tube below the insertion of the stamens) are present in *Pottsia* and some species of *Trachelospermum* and *Epigynum* Wight, and basal collars are found in *Amalocalyx* Pierre, *Epigynum*, and *Sindechites* Oliv. (Middleton, 2005a, in press).

Relationships within the Asian clade are well resolved but with varying levels of support. There are a number of implications for generic delimitations (see Appendix 4 for a list of genera resurrected as a result of this study). *Micrechites* Miq., recently synonymized with *Ichnocarpus* R. Br. (Forster, 1992; Middleton, 1994) and represented here by *I. polyanthus* (Blume) P. I. Forst., *I. rhombifolius* (Markgr.) D. J. Middleton, and *I. serpyllifolius* (Blume) P. I. Forst., is well separated from *Ichnocarpus* s. str. (*I. frutescens* (L.) W. T. Aiton). *Aganosma marginata* (Roxb.) G. Don does not form a monophyletic group with the rest of the genus and should be removed to *Amphineurion* (A. DC.) Pichon as was suggested by Pichon (1950a). Both *Micrechites* and *Amphineurion* can be defined by clear

suites of morphological characters not considered as sufficiently diagnostic by recent workers. Middleton (2006) has now reinstated both genera. *Sindechites* is likewise resolved as polyphyletic, with *S. henryi* Oliver strongly supported as sister to *Aganosma marginata* (98% BS) and *S. chinensis* (Merr.) Markgr. & Tsiang moderately supported (62% BS) as sister to a large clade that includes *Urceola* Roxb. and *Epigynum*. The circumscription of *Urceola* was recently much expanded (Middleton, 1995, 1996b); the present topology indicates that it may need to be further expanded to encompass *Parameria*, regarded by Middleton (1995) as closely related. *Epigynum* is also resolved as paraphyletic, but without bootstrap support.

Apocynum is well nested within the Asian clade and strongly supported as sister to *Cleghornia* Wight (99% BS), confirming the hypothesis of Nilsson et al. (1993) that the tetrads (char. 12, 1) in this genus are convergent with those of Periplocoideae (Fig. 1B, C).

NEW WORLD CLADE (FIG. 1D)

The New World clade is weakly supported (58% BS). Previous studies with broad sampling among members of the APSA clade have not resolved a similar clade (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002), but Simões et al. (2004) found a congruent clade with much narrower sampling. All taxa in our New World clade are endemic to the Americas except for the two predominantly Australasian genera *Artia* Guillaumin and *Parsonsia* R. Br.; *Parsonsia* also extends into tropical and subtropical Asia (Boiteau, 1981; Williams, 1996; Middleton, 1997; Li & Huang, 1998; Heads & de Lange, 1999). The present study does not identify any synapomorphies for the New World clade.

Pichon (1950a) included most of the genera of the New World clade in Echiteae. He placed *Odontadenia* Benth. and *Secondatia* A. DC. in Apocynae, and *Elytropus* Müll. Arg., *Forsteronia* G. Mey., *Mesechites* Müll. Arg. (including *Allomarkgrafia* Woodson [Pichon, 1950a]), and *Mandevilla* (including *Macrosiphonia* Müll. Arg. [Pichon, 1948a]) in Mesechiteae. Leeuwenberg (1994) included the entire New World clade in his Echiteae, except *Forsteronia*, which he transferred to Apocynae.

Endress and Bruyns (2000) largely concurred with Pichon (1950a) in their classification of most taxa of the New World clade, although they followed Leeuwenberg (1994) in retaining *Forsteronia* in Apocynae, where they also moved *Elytropus* and *Trachelospermum* (including *T. difforme*). Finally, they transferred *Secondatia* and *Galactophora* to Mesechiteae.

Simões et al. (2004, 2006, 2007a) concurred with Pichon's exclusion of *Galactophora* and *Secondatia* from Mesechiteae and with the inclusion of *Macrosiphonia* in *Mandevilla*. They identified the distinctive retinacle, formed by cellular fusion rather than adhesion via trichomes (char. 6, 1), the five-ribbed style-head, truncate basal anther appendages, and presence of foliar colleters (char. 2, 1) as synapomorphies of Mesechiteae. They also resolved *Secondatia* and *Odontadenia* as sister taxa, returning the former to Apocynae.

Morales (2003) found the "spool-shaped" style-head of *Secondatia* consistent with tribe Echiteae, a conclusion congruent with its position in the New World clade but outside the *Mesechites* subclade.

The resolution of *Elytropus* as sister to the *Mesechites* subclade, albeit without bootstrap support, is intriguing given Pichon's inclusion of it in Mesechiteae (Pichon, 1950a). However, this unusual monotypic genus, endemic to the temperate rainforests of Chile and Argentina (Ezcurra, 1999), has none of the synapomorphies of Mesechiteae. It lacks foliar colleters and has acute basal anther appendages, a retinacle of agglutinated trichomes, and a pentagonal style-head in cross section.

Many relationships among the major clades within the New World clade are not resolved or are unsupported, but the diversity of retinacle structures and style-head shapes in the group points to previously unsuspected lability of these characters.

The present study resolves *Forsteronia* as polyphyletic, with *F. corymbosa* (Jacq.) G. Mey. (not sampled by Simões et al. [2004]) forming a strongly supported clade with *Trachelospermum difforme* (100% BS). Hansen (1985) recognized the distinctiveness of *F. corymbosa* and of three congeners from Central America and the Caribbean, erecting the invalid subgenus *Pinochia* for them. These four species lack foliar colleters, have acute basal anther appendages, and have a basal collar on the style-head; their retinacle has not been studied. The inclusion of these species in *Forsteronia* is probably due to the similar appearance of their small flowers, with almost completely exerted stamens, a condition otherwise quite rare in New World Apocynoideae. The present topology would be congruent either with treating these species as congeneric with *T. difforme* or erecting a new genus for them.

Trachelospermum difforme, endemic to the southeastern United States, was initially described in *Echites* P. Browne and transferred by various authors to *Forsteronia*, *Secondatia*, and the illegitimate *Thyrsanthus* Benth. (see Krings, 2003). Gray (1878: 85) placed it "somewhat dubiously" in the Asian genus *Trachelospermum*, probably based on the bio-

geographic affinity between the southeastern United States and East Asia. Pichon (1948c) discussed the morphological differences between Asian and American *Trachelospermum* and erected the monotypic genus *Thyrsanthella* (Baill.) Pichon for the latter by elevating *Forsteronia* section *Thyrsanthella* Baill. to generic rank. However, *T. difforme* has been retained in *Trachelospermum* in the floristic literature (e.g., Rosatti, 1989), a position no longer tenable given the results of the present study (Fig. 1C, D).

Our results also have implications for a number of other generic circumscriptions. Morales (1998) synonymized *Salpinctes* Woodson (here resolved as sister to *Pentalinon* Voigt–*Angadenia* Miers [100% BS]) with *Mandevilla*, although it has none of the synapomorphies of Mesechiteae, lacking foliar colleters and possessing acute basal anther appendages, a visor retinacle, and a style-head with a basal collar. Combined with its position in the present analysis, this is strong evidence for recognizing it as a distinct genus.

Prestonia R. Br. is resolved as polyphyletic, with most of the species sampled here grouping with *Artia* and *Parsonsia* (100% BS), whereas *Prestonia riedelii* (Müll. Arg.) Markgr. is sister to *Rhodocalyx* (77% BS) in a separate clade. Ezcurra et al. (1992) pointed out the many similarities between *Prestonia riedelii* and the monotypic *Rhodocalyx*, which led Morales (1999b) to transfer *Rhodocalyx* to *Prestonia*. Our results highlight the need to reappraise *Prestonia*, since its only diagnostic character is the presence of an annulus in the throat of the corolla (Woodson, 1936; Morales, 1997b). *Laubertia* A. DC. has also been associated with *Prestonia* on the basis of this feature (Woodson, 1931; Morales, 2002) but occupies a position separate from all *Prestonia* species in our analysis.

The Australasian genera *Parsonsia* and *Artia* are nested within a paraphyletic (albeit unsupported) grade of species of the predominantly South American *Prestonia*. These three genera share the presence of syncarpous fruits (found in all *Parsonsia* and *Artia* and some *Prestonia* species) and clear latex. A number of additional taxa will need to be sampled to draw firmer conclusions about the closest relatives of *Artia* and *Parsonsia* in the New World: *Hylaea* J. F. Morales (Morales, 1999a), *Thoreauea* J. K. Williams (Williams, 2002), and *Thenardia* Kunth (Williams, 1998, 1995) from the New World, *Ecua* D. J. Middleton (Middleton, 1996a) from Malesia, as well as a larger sample of *Prestonia* and *Parsonsia* species. Our results confirm Middleton's (1997) decision to expand the circumscription of *Parsonsia* to include the monotypic *Delphyodon* K. Schum. (*P. oligantha* (K. Schum.) D. J. Middleton in Fig. 1D). *Artia*, endemic to

New Caledonia after the removal of the Asian species to *Parsonsia* (see Middleton, 1997), bears many similarities to the latter genus. In our results, *Artia* is sister to a monophyletic *Parsonsia* (92% BS), but the sampling in *Parsonsia*, a genus of ca. 82 species, remains low.

The circumscription of *Echites* has varied enormously (Woodson, 1933, 1936; Morales, 1997a). Most recently, Morales and Williams (2004) erected a new genus, *Allotoonia* J. F. Morales & J. K. Williams, for the five species of *Echites* subgenus *Pseudechites* Woodson and restricted *Echites* s. str. to the four species of subgenus *Echites*, a decision based on a cladistic analysis of morphological characters that resolved the two subgenera of *Echites* as polyphyletic with subgenus *Echites* sister to *Fernaldia* Woodson and subgenus *Pseudechites* sister to *Thenardia* (not sampled here; Williams, 2004). The present study supports inclusion of *Allotoonia* in *Echites* s.l. and the expansion of the circumscription of *Echites* to include *Fernaldia* with high bootstrap values (99%–100% BS). The affinity between *Fernaldia* and *Echites* and the paucity of characters separating the two genera has been noted by Williams (2004). Characters used to separate *Echites* s. str. and *Allotoonia* include visibility of tertiary leaf venation, inflorescence branching, corolla lobe shape, and latex color, milky in *Echites* s. str. versus clear in *Allotoonia* (Morales & Williams, 2004). However, the latex of *E. umbellatus* Jacq., which groups with the species segregated into *Allotoonia*, has been reported as both clear and milky on herbarium labels, and living plants from the Bahamas and southern Florida were always found to have clear latex (E. Fried, B. Rathke, S. Zona, pers. comm.).

RHABDADENIA (FIG. 1B)

Our analysis places *Rhabdadenia*, a Caribbean and South American genus of four species (Ezcurra et al., 1992) in a polytomy with the New World clade and the African clade–Secamonoideae–Asclepiadoideae. This genus has been consistently included in Echiteae, but Pichon (1948b) regarded its retinacle as unusually reduced to a weak longitudinal nonfunctional rib with the anthers adhering to the style-head only by the bases of the anther thecae. However, there is a tuft of trichomes at the base of the anther that appears to be weakly attached to the style-head, rather similar to a brush retinacle.

AFRICAN CLADE (FIG. 1B)

The African clade, composed of *Baissea* (18 species)–*Motandra* (3 species)–*Oncinotis* (7 species),

is strongly supported as monophyletic (100% BS). Its greatest species diversity is in tropical West Africa, but one species of *Oncinotis* is endemic to Madagascar (Markgraf, 1976; De Kruif, 1983, 1985; Van Dilst, 1995). These genera have unique branched trichomes and leaves with domatia, which are likely synapomorphies (Omino, 1996).

Pichon (1950a) included these genera in Mesechi-teae as subtribe Baisseinae based on his misinterpretation of their retinacles as glabrous facets (Table 2) and on the presence of colleters on the leaves. Nilsson et al. (1993) investigated floral anatomy of *Baissea* and concluded that the retinacle is formed via adhesion by trichomes. We have confirmed this for representatives of all three genera of the African clade. Both Leeuwenberg (1994) and Endress and Bruyns (2000) included these three genera in their Apocynaeae on the basis of several characters that occur frequently in the Asian clade, including the very short stamen–corolla tube, half-inferior ovaries, and collarless style-head. These characters are also present in Periplocoideae, Secamonoideae, and Asclepiadoideae and may be symplesiomorphies for the crown clade. *Baissea* has frequently been associated with the Asian *Cleghornia*, which Bentham (1876) treated as a synonym (see Macfarlane, 1933; Xu, 1988).

AFRICAN CLADE SECAMONOIDEAE–ASCLEPIADOIDEAE (FIG. 1B)

The African clade is strongly supported as sister to Secamonoideae–Asclepiadoideae (93% BS), a result corroborated by Lahaye et al. (2007) and Simões et al. (2007b). Various hypotheses of relationships between *Baissea* and these two subfamilies have been resolved in previous analyses, but always without support (Sennblad et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002).

The presence of colleters on the leaves (char. 2, 1) is unambiguously optimized as a synapomorphy for the African clade and Secamonoideae–Asclepiadoideae. However, in the African clade, colleters are predominantly distributed along the adaxial surface of the petiole and occasionally extend onto the base of the lamina, whereas in Secamonoideae and Asclepiadoideae, they are on the base of the lamina and do not extend along the petiole.

Prior to the study of Sennblad et al. (1998), only Macfarlane (1933) had suggested that *Baissea* and *Oncinotis* might be most closely related to Secamonoideae–Asclepiadoideae, although he did not identify colleters on the petiole as diagnostic for this group. In fact, the characters he cited in his argument for these genera as ancestral to Secamonoideae–Asclepiadoideae are mostly widespread in the crown

clade or were misinterpreted by him. Macfarlane probably reached this conclusion because of his interest in geographic distributions and centers of origin. Most of the approximately 200 species of Secamonoideae are endemic to Africa and Madagascar (Klackenberg, 1992).

EVOLUTION OF COMPLEX POLLINATION MECHANISMS (FIG. 1B)

Asclepiadaceae (Periplocoideae and Secamonoideae–Asclepiadoideae) are here resolved as polyphyletic, although there is no bootstrap support for the node that precludes a paraphyletic Asclepiadaceae. The characters that have been interpreted as evidence for their sister group relationship (retinacle formed by cellular fusion [char. 6, 1], presence of a translator [char. 7, 1], nectar secretion from staminal feet or a fused basal tube [char. 8, 2], pollen dispersed in tetrads [char. 12, 1]) are reconstructed as parallelisms. The presence of pollinia (char. 13, 1) and of a fused basal tube (char. 4, 1) are reconstructed as parallelisms for Secamonoideae–Asclepiadoideae and certain genera of Periplocoideae. The most recent common ancestor (MRCA) of the African clade–Secamonoideae–Apocynoideae is reconstructed as having pollen in aperturate monads, a foamy style-head adhesive that is not molded into translators, no fused basal tube, and a nectary disk around the ovary, whereas the MRCA of Secamonoideae–Asclepiadoideae is reconstructed as having inaperturate pollen aggregated into four pollinia, translators, and anthers inserted on a fused basal tube with the tissue of alternistaminal sectors of the tube nectariferous. In other words, there are no extant representatives of lineages that evolved along the branch between the MRCA of the African clade–Secamonoideae–Asclepiadoideae (with flowers that are reconstructed as indistinguishable from most other crown clade Apocynoideae) and the MRCA of Secamonoideae–Asclepiadoideae (with flowers that are reconstructed as indistinguishable from modern Secamonoideae).

Simões et al. (2007b), using a much smaller sample of APSA clade taxa, found weak support for a paraphyletic Asclepiadaceae. According to their topology, a retinacle formed by cellular fusion (char. 6, 1), presence of a translator (char. 7, 1), nectar secretion from staminal feet or a fused basal tube (char. 8, 2), and pollen dispersed in tetrads (char. 12, 1) could be reconstructed with equal parsimony as parallelisms or as symplesiomorphies shared by Periplocoideae and Secamonoideae–Asclepiadoideae. If the latter reconstruction is preferred, these characters were secondarily lost in the African clade lineage.

The fossil record yields no additional clues. Konzalova (1981) described fossil massulae, *Asclepia-*

diopollis chebensis, from strata of unknown but suspected Paleogene age from Czechoslovakia. The massulae are aggregates of four tetrahedral to polygonal tetrads with a faintly granular exine and one to three indistinct pores per pollen grain. Although Konzalova (1981) interpreted *A. chebensis* as representing a taxon transitional between Periplocoideae and Secamonoideae, the fossils match quite well the pollen morphology of pollinia-bearing Periplocoideae (represented here by *Gymnanthera* R. Br. and *Finlaysonia* Wall. [Fig. 1B]), which tend to have highly reduced pores in the outer walls of the pollinia (Verhoeven & Venter, 1998, 2001).

BIOGEOGRAPHY (FIG. 1A–D)

Our results demonstrate a stronger correlation between phylogeny and geographic distribution in the APSA clade than suggested by previous classifications of Apocynoideae. The *Wrightia* and *Nerium* clades are exclusively Old World (Fig. 1A). Within the crown clade, Apocynoideae are divided into five geographically structured lineages: the Asian clade (with one New World subclade; Fig. 1C), the New World clade (with one Australasian subclade; Fig. 1D), the New World *Rhabdadenia* (Fig. 1B), and the African clade (Fig. 1B). Two other crown clade lineages, Periplocoideae and Secamonoideae, are exclusively Old World (Fig. 1B). Only the *Malouetia* clade (Fig. 1B) and Asclepiadoideae (Fig. 1B) have multiple subclades with representatives in both the New and Old Worlds. The *Malouetia* clade has two subclades with sister taxa in Africa and the New World: *Neobraccia* (Caribbean)–*Pachypodium* (southern Africa and Madagascar) and *Malouetia* (distributed in tropical West Africa and Central and South America; Fig. 1B). *Galactophora*, sister to the rest of the *Malouetia* clade, is endemic to tepuis and white sand areas of the Guyana Shield in South America (Zarucchi et al., 1995). Three subclades of Asclepiadoideae have both Old and New World representatives: *Cynanchum* L., *Marsdenia*, and the subtribe Asclepiadinae (Rapini et al., 2003); all other New World Asclepiadoideae are part of the exclusively New World Metastelmatinae, Oxypetalinae, and Gonolobinae (MOG) clade (Rapini et al., 2003; Liedt-Schumann et al., 2005).

The fossil record of the APSA clade is very poor and does not aid in reconstructing the past geographical distributions of its subclades. Only Periplocoideae and *Apocynum* (Asian clade) are known from unambiguous fossils. Fossil pollen of two periplocoid genera, *Tacazzea* Decne. and *Periploca* L., has been reported from the Oligocene and lower Miocene from Cameroon and the Miocene from Eurasia, respective-

ly. Fossil pollen of *Apocynum* has been reported from the upper Miocene from Spain (Muller, 1981). These occurrences are within the current ranges of these genera. Fossil comose seeds from middle to late Eocene North America and middle Eocene to early Oligocene Europe (Manchester, 1999) belong to the APSA clade but cannot be classified with any more taxonomic precision given the absence of diagnostic seed characters for most APSA clade lineages.

CONCLUSIONS

This study represents a major advance in the understanding of phylogenetic relationships within the APSA clade, placing representatives of 59 of 77 currently recognized genera of Apocynoideae in context with exemplars of Periplocoideae, Secamonoideae, and Asclepiadoideae (Fig. 1A, B). The Apocynoideae and their tribes are non-monophyletic as delimited in the classifications of Pichon (1950a), Leeuwenberg (1994), and Endress and Bruyns (2000) as amended by Simões et al. (2004; Fig. 1A–D).

We have identified nested sets of synapomorphies that support relationships among the *Wrightia*, *Nerium*, *Malouetia*, and crown clades (Fig. 1A, B). The *Wrightia* clade retains the plesiomorphic states of self-supporting growth form, sinistrorsely contorted corollas, absence of a nectary, and absence of a micropylar coma; the rest of the APSA clade shares the synapomorphies of dextrorsely contorted corollas and presence of a micropylar coma. The sister relationship between the *Malouetia* clade and the crown clade is supported by the shared presence of a nectary. The crown clade lineages share the synapomorphic presence of dependent growth form. Although parallelisms and/or reversals have occurred in all of these characters (Fig. 1A, B), combined with features of the retinacle, style-head, chalazal coma, and foliar colleters, and also with biogeography (Fig. 1A, B), they are sufficient to diagnose each of the seven primary clades of Apocynoideae identified here.

At this point, the paraphyly of Apocynoideae with respect to Periplocoideae, Secamonoideae, and Asclepiadoideae has been corroborated by numerous studies (Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Sennblad et al., 1998; Potgieter & Albert, 2001; Simões et al., 2007b; this study). If the latter three taxa are maintained as subfamilies in a strictly monophyletic classification of Apocynaceae s.l., it would be necessary to divide Apocynoideae into no fewer than seven subfamilies, corresponding to the *Wrightia*, *Nerium*, *Malouetia*, *Rhabdadenia*, Asian, New World, and African clades (Fig. 1A, B). This number could be reduced if *Rhabdadenia* is treated as incertae sedis and/or if Secamonoideae are reduced to

a tribe of Asclepiadoideae. Whether a strictly monophyletic classification of the APSA clade is desirable and how its constituent groups should be circumscribed will be the subject of future papers.

The non-monophyly of Asclepiadaceae and the sister group relationship of the African clade and Secamonoideae–Asclepiadoideae (strongly supported for the first time in three studies published in this symposium volume [Lahaye et al., 2007; Simões et al., 2007b; this study]) have profound implications for our understanding of the evolution of pollination mechanisms in the Apocynaceae. Of the three proposed scenarios, that of MacFarlane (1933) best fits our phylogenetic result (Fig. 1); pollen aggregation and mass transfer have evolved independently multiple times within the APSA clade. Pollen aggregated into tetrads is reconstructed as evolving at least three times independently: in *Apocynum*, Periplocoideae, and the MRCA of Secamonoideae–Asclepiadoideae (Fig. 1B, C). Pollinia are reconstructed as evolving a minimum of two times independently (once in the MRCA of Secamonoideae–Asclepiadoideae and at least once within Periplocoideae; Fig. 1B, C). Other studies indicate that pollinia have arisen more than once within Periplocoideae (Verhoeven & Venter, 2001; Fishbein, 2001; Ionta & Judd, 2007).

The results presented here indicate that we cannot reconstruct a detailed evolutionary transformation series leading from the flowers of Apocynoideae to those of Secamonoideae. The taxa with intermediate morphologies are extinct and not known from the fossil record. However, the paraphyletic Asclepiadaceae recovered by Simões et al. (2007b) with a much sparser sample of the APSA clade leaves open the possibility that Periplocoideae are indeed the intermediate between Apocynoideae and Secamonoideae and that translators and tetrads were secondarily lost in the African clade. The position of Periplocoideae needs to be clarified by incorporating novel sources of data (e.g., nuclear and mitochondrial genes) in a densely sampled analysis of the APSA clade so that the homologies of the highly modified floral structures of Periplocoideae and Secamonoideae–Asclepiadoideae can be interpreted in the context of a well-supported phylogeny. Future research also needs to focus on identification of organismic synapomorphies for most of the clades reconstructed in this study.

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Appendix 1. Voucher data and GenBank accession numbers. Previously published sequences are cited according to the following code: A = Simões et al. (2004); B = Simões et al. (2007b); B* = identified as *Cerbera venenifera* (Poir.) Steud. in Simões et al. (2007b). Subfamilial and tribal classification is according to Endress and Bruyns (2000) as amended by Endress and Stevens (2001) and Simões et al. (2004). Generic classification of Apocynoideae is that of Endress and Bruyns (2000) as amended by Simões et al. (2004, 2006, 2007a) and Morales and Williams (2004). Generic classification of Periplocoideae is according to Venter and Verhoeven (2001).

Subfamily, Tribe	Genus species Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer; <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
Apocynoideae, Apocyneae	<i>Aganosma cymosa</i> (Roxb.) G. Don	Thailand, D. J. Middleton 243 (A)	EF456144; EF456608; EF456496; EF456274
	<i>Aganosma cymosa</i> (Roxb.) G. Don	Thailand, D. J. Middleton et al. 1101 (A)	EF456120; EF456605; EF456389; –
	<i>Aganosma marginata</i> (Roxb.) G. Don	Thailand, D. J. Middleton et al. 2078 (A)	EF456125; EF456544; EF456491; EF456258
	<i>Aganosma schlechteriana</i> H. Lév.	Thailand, Newman et al. 1069 (A)	EF456126; EF456606; EF456494; EF456259
	<i>Aganosma wallichii</i> G. Don	Thailand, D. J. Middleton et al. 1424 (A)	EF456145; EF456609; EF456497; –
	<i>Aganosma wallichii</i> G. Don	Thailand, D. J. Middleton et al. 1459 (A)	EF456127; EF456607; EF456495; EF456260
	<i>Amalocalyx microlobus</i> Pierre ex Spire	Thailand, D. J. Middleton 211 (A)	EF456128; EF456545; EF456505; EF456261
	<i>Anodendron oblongifolium</i> Hemsl.	Papua New Guinea, W. Takeuchi 14513 (A)	EF456123; EF456543; EF456419; EF456256
	<i>Anodendron paniculatum</i> A. DC.	Thailand, D. J. Middleton et al. 3159 (A)	EF456194; EF456618; EF456499; EF456315
	<i>Apocynum androsaemifolium</i> L.	U.S.A., New York, T. Livshultz 03-32 (BH, GH)	EF456130; EF456546; EF456387; EF456263
	<i>Apocynum cannabinum</i> L.	U.S.A., New York, T. Livshultz 03-28 (BH)	EF456131; B; B; B
	<i>Baisea multiflora</i> A. DC.	cult., Belgium, Nat. Bot. Gard. Belgium #74-3527, F. Billiet S3853 (BR)	EF456199; EF456620; EF456451; EF456319
	<i>Beaumontia grandiflora</i> Wall.	cult., Germany, Bot. Gard. Munich, G. Gerlach & J. Babczinsky 5/06 (M)	EF456184; EF456556; EF456498; EF456306
	<i>Chonemorpha fragrans</i> (Moon) Alston	Thailand, D. J. Middleton 220 (A)	EF456132; EF456547; EF456489; EF456264
	<i>Cleghornia malaccensis</i> (Hook. f.) King & Gamble	China, Tsi Zhanhuo 92-387 (MO)	EF456241; EF456622; EF456504; EF456356
	<i>Elytropus chilensis</i> (A. DC.) Müll. Arg.	Chile, M. Mihoc et al. Herb. Conc. #156934 (CONC)	EF456228; EF456614; EF456445; EF456307
	<i>Epigynum auritum</i> (C. K. Schneid.) Tsiang & P. T. Li	Thailand, D. J. Middleton et al. 1073 (A)	EF456121; EF456540; EF456503; EF456253
	<i>Epigynum auritum</i> (C. K. Schneid.) Tsiang & P. T. Li	Thailand, D. J. Middleton et al. 1457 (A)	EF456146; EF456567; EF456507; –
	<i>Epigynum cochinchinense</i> (Pierre) D. J. Middleton	Thailand, D. J. Middleton 209 (A)	EF456147; EF456568; EF456508; EF456275
	<i>Epigynum cochinchinense</i> (Pierre) D. J. Middleton	Thailand, D. J. Middleton et al. 928 (A)	EF456133; EF456548; EF456506; EF456265
	<i>Epigynum griffithianum</i> Wight	Thailand, D. J. Middleton et al. 1934 (A)	EF456227; EF456626; EF456514; EF456344
	<i>Epigynum ridleyi</i> King & Gamble	Indonesia, Kalimantan, Ambriansyah & Arifin 315 (I)	EF456245; EF456639; EF456515; EF456359
	<i>Ichnocarpus frutescens</i> (L.) W. T. Aiton	Australia, P. I. Forster 26539 (BRI)	EF456136; EF456552; EF456490; EF456267
	<i>Ichnocarpus polyanthus</i> (Blume) P. I. Forst.	Thailand, D. J. Middleton et al. 1950 (A)	EF456225; EF456624; EF456511; EF456342
	<i>Ichnocarpus rhombifolius</i> (Markgr.) D. J. Middleton	Papua New Guinea, W. Takeuchi & Ama 15692 (A)	EF456190; EF456588; EF456471; –
	<i>Ichnocarpus serpyllifolius</i> (Blume) P. I. Forst.	Brunei, D. J. Middleton et al. 779 (A)	EF456137; EF456553; EF456472; EF456268

Appendix 1. Continued.

Subfamily, Tribe	Genus species Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer; <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
Apocynoideae, Echiteae	<i>Motandra guineensis</i> (Tonn.) A. DC.	Ivory Coast, <i>Markin & Pascal s.n.</i> (Z)	EF456210; EF456621; EF456411; EF456333
	<i>Odontadenia lutea</i> (Vell.) Markgr.	Brazil, <i>L. S. Kinoshita 2002/56</i> (UEC)	EF456220; A: –; EF456337
	<i>Odontadenia perrottetti</i> (A. DC.) Woodson	French Guiana, <i>Prévost & Feuillet 3973</i> (CAY)	EF456140; EF456554; EF456427; EF456272
	<i>Odontadenia puncticulosa</i> (Rich.) Pulle	French Guiana, <i>Prévost 3713</i> (CAY)	EF456187; EF456557; EF456488; EF456310
	<i>Oncinotis tenuiloba</i> Stapf	South Africa, <i>T. Abbott 7707</i> (Z)	EF456141; B; B; B
	<i>Papuechites aambe</i> (Warb.) Markgr.	Papua New Guinea, <i>Takeuchi & Ama 16124</i> (A)	EF456189; EF456587; EF456448; EF456312
	<i>Parameria laevigata</i> (Juss.) Moldenke	Thailand, <i>D. J. Middleton et al. 2551</i> (A)	EF456197; EF456598; EF456513; EF456368
	<i>Pottsia laxiflora</i> (Blume) Kuntze	Thailand, <i>D. J. Middleton et al. 388</i> (A)	EF456149; EF456570; EF456493; EF456276
	<i>Secondatia densiflora</i> A. DC.	Brazil, <i>A. Simões et al. 1218</i> (UEC)	EF456177; EF456564; EF456440; EF456299
	<i>Sindechites chinensis</i> (Merr.) Markgr. & Tsiang	Thailand, <i>Newman et al. 1034</i> (A)	EF456151; EF456572; EF456492; EF456278
	<i>Sindechites henrvi</i> Oliv.	China, <i>Sino-American Guizhou Botanical Expedition 310</i> (A)	EF456244; EF456638; EF456502; EF456367
	<i>Trachelospermum asiaticum</i> (Sieb. & Zucc.) Nakai	cult., Belgium, Nat. Bot. Gard. Belgium #56-2159, <i>F. Billiet S218</i> (BR)	EF456207; EF456595; EF456517; EF456324
	<i>Trachelospermum asiaticum</i> (Sieb. & Zucc.) Nakai	cult., Belgium, Nat. Bot. Gard. Belgium #98-1661-49, <i>V. Leyman S3857</i> (BR)	EF456204; EF456604; EF456516; EF456322
	<i>Trachelospermum asiaticum</i> (Sieb. & Zucc.) Nakai	cult., U.S.A., Cornell University, <i>T. Livshultz 03-25</i> (A, BH)	EF456155; EF456573; EF456501; EF456281
	<i>Trachelospermum axillare</i> Hook. f.	Thailand, <i>Suksathan & D. J. Middleton 1852</i> (A)	EF456156; EF456574; EF456509; EF456282
	<i>Trachelospermum difforme</i> (Walter) A. Gray	cult., U.S.A., Florida, <i>G. Ionta 460</i> (FLAS)	EF456171; EF456562; EF456435; EF456293
	<i>Trachelospermum jasminoides</i> (Lindl.) Lem.	cult., U.S.A., Univ. Florida, <i>G. Ionta 420</i> (FLAS)	EF456172; EF456578; EF456436; EF456294
	<i>Urceola lucida</i> (Wall. ex G. Don) Benth. ex Kurz	Thailand, <i>D. J. Middleton et al. 2058</i> (A)	EF456226; EF456625; EF456512; EF456343
	<i>Urceloa micrantha</i> (Wall. ex G. Don) D. J. Middleton	Thailand, <i>D. J. Middleton et al. 1003</i> (A)	EF456157; EF456575; EF456510; EF456283
	<i>Urceola minutiflora</i> (Pierre) D. J. Middleton	Thailand, <i>D. J. Middleton 193</i> (A)	EF456124; –; –; –
	<i>Urceola minutiflora</i> (Pierre) D. J. Middleton	Laos, <i>D. J. Middleton & Lamxay 287</i> (A)	EF456158; EF456576; EF456385; –
	<i>Urceola minutiflora</i> (Pierre) D. J. Middleton	Laos, <i>D. J. Middleton & Lamxay 255</i> (A)	EF456159; –; –; EF456284
	<i>Urceola rosea</i> (Hook. & Arn.) D. J. Middleton	Thailand, <i>D. J. Middleton et al. 481</i> (A)	EF456160; EF456603; EF456386; EF456285
	<i>Vallaris solanacea</i> (Roth) Kuntze	Thailand, <i>D. J. Middleton et al. 1704</i> (A)	EF456162; EF456559; EF456500; EF456286
	<i>Allotoonia agglutinata</i> (Jacq.) J. F. Morales & J. K. Williams	Puerto Rico, <i>C. M. Taylor 8079</i> (NY)	EF456232; EF456630; EF456460; EF456348
	<i>Allotoonia woodsoniana</i> (Monach.) J. F. Morales & J. K. Williams	Costa Rica, <i>F. Morales 8810</i> (INB)	EF456175; EF456580; EF456403; EF456297

Subfamily, Tribe	Genus species Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer: <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
	<i>Angadenia berteroi</i> (A. DC.) Miers	Cuba, S. Nilsson s.n. (Z)	EF456129; EF456611; EF456416; EF456262
	<i>Angadenia sagraei</i> Miers	Bahamas, M. Vincent 11459 (GH)	EF456182; EF456613; EF456443; EF456304
	<i>Artia balansae</i> (Baill.) Pichon ex Guillaumin	New Caledonia, G. McPherson et al. 19257 (MO)	EF456209; EF456534; EF456481; EF456325
	<i>Cycladenia humilis</i> Benth.	U.S.A., California, B. Smith 822 (Z)	EF456211; EF456558; EF456397; EF456334
	<i>Echites turriger</i> Woodson	Mexico, W. P. Spencer & J. K. Williams 2 (SHST)	EF456196; EF456590; EF456404; EF456317
	<i>Echites umbellatus</i> Jacq.	Bahamas, M. Vincent 11468 (GH)	EF456181; EF456584; EF456390; EF456303
	<i>Echites umbellatus</i> Jacq.	Mexico, R. Orellana 975 (NY)	EF456237; EF456633; –; EF456352
	<i>Fernaldia pandurata</i> (A. DC.) Woodson	Costa Rica, L. Gomez s.n. (Z)	EF456191; EF456560; EF456401; EF456313
	<i>Laubertia contorta</i> (M. Martens & Galeotti) Woodson	cult., Sam Houston State University, J. Williams 2005-1 (SHST)	EF456246; EF456640; EF456469; EF456360
	<i>Neobracea bahamensis</i> (Britton) Britton	Bahamas, M. Vincent 11466 (GH)	EF456183; EF456585; EF456444; EF456305
	<i>Neobracea ekmannii</i> Urb.	Cuba, S. Nilsson s.n. (Z)	EF456176; EF456581; EF456439; EF456298
	<i>Neobracea valenzuelana</i> (A. Rich.) Urb.	Cuba, S. Nilsson s.n. (Z)	EF456139; EF456565; EF456426; EF456270
	<i>Parsonsia crebriflora</i> Baill.	New Caledonia, G. McPherson and J. Munzinger 18220 (MO)	EF456214; EF456535; EF456482; EF456327
	<i>Parsonsia eucalyptophylla</i> F. Muell.	Australia, P. I. Forster 20118 (BRI)	EF456142; B; B; B
	<i>Parsonsia ferruginea</i> J. B. Williams	Australia, B. Hyland 16142 (QRS)	EF456219; EF456538; EF456486; EF456335
	<i>Parsonsia flexuosa</i> Baill.	New Caledonia, G. McPherson & J. Munzinger 18046 (MO)	EF456218; EF456537; EF456485; EF456332
	<i>Parsonsia lata</i> Markgr.	Papua New Guinea, Takeuchi & Ama 16379 (A)	EF456188; EF456533; EF456428; EF456311
	<i>Parsonsia longiflora</i> Guillaumin	New Caledonia, G. McPherson & J. Munzinger 18151 (MO)	EF456216; EF456536; EF456483; EF456330
	<i>Parsonsia oligantha</i> (K. Schum.) D. J. Middleton	Papua New Guinea, W. Takeuchi 14502 (A)	EF456143; EF456532; EF456429; EF456273
	<i>Peltastes isthmicus</i> Woodson	Costa Rica, M. E. Endress 97-16 (Z)	EF456179; EF456583; EF456441; EF456301
	<i>Peltastes peltatus</i> (Vell.) Woodson	cult., Belgium, Nat. Bot. Gard. Belgium #84-2849, F. Billiet S3526 (BR)	EF456203; B; B; B
	<i>Pentalinon luteum</i> (L.) B. F. Hansen & Wunderlin	Bahamas, M. Vincent 11460 (GH)	EF456180; EF456612; EF456442; EF456302
	<i>Prestonia coalita</i> (Vell.) Woodson	Brazil, L. S. Kinoshita et al. 2000/10 (UEC)	EF456185; EF456586; EF456447; EF456309
	<i>Prestonia lagoensis</i> (Müll. Arg.) Woodson	Paraguay, E. M. Zardini & L. Guerrero 44456 (AS, MO)	EF456215; EF456601; EF456455; EF456329
	<i>Prestonia mexicana</i> A. DC.	Mexico, E. Martinez et al. 29350 (NY)	EF456229; EF456627; EF456487; EF456345
	<i>Prestonia portobellensis</i> (Beurl.) Woodson	Mexico, F. Ventura 21262 (NY)	EF456235; EF456631; EF456462; EF456350

Appendix 1. Continued.

Subfamily, Tribe	Genus species Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer; <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
Apocynoideae, Malouetieae	<i>Prestonia quinquangularis</i> (Jacq.) Spreng.	cult., Belgium, Nat. Bot. Gard. Belgium #51-0287, F. Billiet S205 (BR)	EF456205; B: B: B
	<i>Prestonia riedelii</i> (Müll. Arg.) Markgr.	Brazil, A. O. Simões 1274 (UEC)	EF456212; A: –; EF456336
	<i>Prestonia riedelii</i> (Müll. Arg.) Markgr.	Bolivia, Solomon & Escobar 12465 (NY)	EF456233; EF456563; EF456461; –
	<i>Prestonia robusta</i> Rusby	Bolivia, Nee & Chavez 51515 (NY)	EF456236; EF456632; EF456463; EF456351
	<i>Prestonia tomentosa</i> R. Br.	Paraguay, E. M. Zardini & W. Cardozo 44964 (AS, MO)	EF456217; EF456602; EF456484; EF456331
	<i>Rhabdadenia biflora</i> (Jacq.) Müll. Arg.	U.S.A., Florida, S. Zona 616 (FTG)	EF456150; EF456571; EF456388; EF456277
	<i>Rhabdadenia macrostoma</i> (Benth.) Müll. Arg.	Peru, Smith et al. 776 (NY)	EF456234; EF456599; –; EF456349
	<i>Rhabdadenia pohlii</i> Müll. Arg.	Paraguay, E. M. Zardini & F. Vieira 41979 (MO, PY)	EF456213; EF456597; EF456480; EF456326
	<i>Rhodocalyx rotundifolius</i> Müll. Arg.	Brazil, L. S. Kinoshita 2000/66 (UEC)	EF456186; EF456561; EF456446; EF456308
	<i>Salpinctes kalmiifolius</i> Woodson	Venezuela, Steyermark et al. 124577 (NY)	EF456231; EF456629; EF456459; EF456347
	<i>Stipecoma peltigera</i> (Stadelm.) Müll. Arg.	Brazil, L. S. Kinoshita s.n. (UEC)	EF456193; EF456616; EF456478; EF456314
	<i>Temnadenia odorifera</i> (Vell.) J. F. Morales	Brazil, A. O. Simões & R. B. Singer 1035 (UEC)	EF456238; EF456634; EF456464; EF456353
	<i>Temnadenia violacea</i> (Vell.) Miers	Brazil, A. O. Simões et al. 144 (UEC)	EF456224; EF456623; EF456479; EF456341
	<i>Alafia barteri</i> Oliv.	Ghana, H. H. Schmidt et al. 2037 (MO)	EF456239; EF456635; EF456465; EF456354
	<i>Alafia thouarsii</i> Roem. & Schult.	Madagascar, G. McPherson et al. 17584 (MO)	EF456242; EF456637; EF456467; EF456357
	<i>Farquharia elliptica</i> (Preuss) Stapf	Ivory Coast, Chatelain et al. 1456 (MO)	EF456242; EF456637; EF456467; EF456357
	<i>Funtumia africana</i> (Benth.) Stapf	cult., Belgium, Nat. Bot. Gard. Belgium #51-4728, V. Leyman S3855 (BR)	EF456206; EF456594; EF456415; EF456323
	<i>Funtumia elastica</i> (Preuss) Stapf	cult., Belgium, Nat. Bot. Gard. Belgium #81-0051, V. Leyman S3927 (BR)	EF456202; B: B: B
	<i>Holarrhena curtisii</i> King & Gamble	Thailand, D. J. Middleton et al. 2042 (A)	EF456122; EF456541; EF456423; EF456254
	<i>Holarrhena curtisii</i> King & Gamble	Laos, D. J. Middleton & Lamxay 253 (A)	EF456148; EF456569; EF456417; –
	<i>Holarrhena pubescens</i> (Buch-Ham.) Wall. ex G. Don	Tanzania, Kuchar & Kidua 25042 (MO)	EF456250; EF456643; EF456475; EF456363
	<i>Holarrhena pubescens</i> (Buch-Ham.) Wall. ex G. Don	Tanzania, Mwangulango & Kipambe 130 (MO)	EF456249; EF456642; EF456474; EF456362
	<i>Holarrhena pubescens</i> (Buch-Ham.) Wall. ex G. Don	Tanzania, Mwiga et al. 39 (MO)	EF456247; EF456641; EF456473; EF456361
	<i>Holarrhena pubescens</i> (Buch-Ham.) Wall. ex G. Don	Thailand, D. J. Middleton 244 (A)	EF456135; EF456551; EF456421; EF456271
	<i>Kibatalia macrophylla</i> (Pierre ex Hua) Woodson	Thailand, D. J. Middleton et al. 1018 (A)	EF456119; EF456542; EF456422; EF456255
	<i>Malouetia bequaertiana</i> Woodson	Cameroun, J. J. F. E. de Wilde 8405 (MO)	EF456243; EF456617; EF456468; EF456358
	<i>Malouetia tamaquarina</i> (Aubl.) A. DC.	French Guiana, S. Mori et al. 25313 (NY)	EF456230; EF456628; EF456457; EF456346
	<i>Mascarenhasia arborescens</i> A. DC.	cult., Belgium, Nat. Bot. Gard. Belgium #85-0085, F. Billiet S3053 (BR)	EF456201; EF456593; EF456453; EF456321

Subfamily, Tribe	Genus species Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer; <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
Apocynoideae, Mesechiteae	<i>Mascarenhasia lisianthiflora</i> A. DC.	Madagascar, <i>Schönenberger et al.</i> A147 (UPS)	EF456174; EF456579; EF456438; EF456296
	<i>Pachypodium baronii</i> Costantin & Bois	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 03-20 (BH)	EF456154; EF456615; EF456449; EF456280
	<i>Pachypodium lamerei</i> Drake	cult., Switzerland, Bot. Gard. Zurich, May 2003, <i>M. E. Endress</i> s.n. (Z)	EF456198; EF456619; EF456458; EF456318
	<i>Spirolobium cambodianum</i> Baill.	Thailand, <i>D. J. Middleton et al.</i> 3181 (A)	EF456195; EF456589; EF456450; EF456316
	<i>Allomarkgrafia brenesiana</i> Woodson	Costa Rica, <i>M. E. Endress</i> 97-06 (Z)	EF456223; A: —; EF456310
	<i>Forsteronia acouci</i> (Aubl.) A. DC.	French Guiana, <i>Prévost</i> 3720 (CAY)	EF456222; A: —; EF456339
	<i>Forsteronia corymbosa</i> (Jacq.) G. Mey.	Puerto Rico, <i>E. Santiago</i> 97-6 (Z)	EF456167; EF456555; EF456432; EF456289
	<i>Forsteronia guyanensis</i> Müll. Arg.	French Guiana, <i>Prévost & Feuillet</i> 3970 (CAY)	EF456134; EF456549; EF456477; EF456266
	<i>Forsteronia relloziana</i> (A. DC.) Woodson	Brazil, <i>Simões</i> 343 (UEC)	EF456221; A: —; EF456338
	<i>Mandevilla boliviensis</i> (Hook. f.) Woodson	cult., U.S.A., Harvard University, <i>T. Livshultz</i> 03-35 (GH)	EF456153; EF456550; EF456425; EF456279
Apocynoideae, Wrighteae	<i>Mandevilla longiflora</i> (Desf.) Pichon	Paraguay, <i>E. M. Zardini & F. Vieira</i> 41973 (MO, PY)	EF456208; EF456600; EF456454; EF456328
	<i>Mesechites trifidus</i> (Jacq.) Müll. Arg.	Ecuador, <i>Liede & Meve</i> 3471 (UBT)	EF456138; EF456566; EF456424; EF456269
	<i>Adenium obesum</i> (Forssk.) Roem. & Schult.	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 03-21 (BH)	EF456152; B: B; B
	<i>Isonema smeathmannii</i> Roem. & Schult.	cult., Belgium, Nat. Bot. Gard. Belgium #64-0527, <i>F. Billiet</i> S1964 (BR)	EF456200; EF456592; EF456452; EF456320
	<i>Verium oleander</i> L.	cult., U.S.A., Univ. Florida, <i>G. Ionta</i> 416 (FLAS)	EF456173; EF456596; EF456437; EF456295
	<i>Pleioceras barteri</i> Baill.	Ghana, <i>Jongkind et al.</i> 2131 (MO)	EF456251; EF456524; EF456476; EF456364
	<i>Stephanostema stenocarpum</i> K. Schum.	Tanzania, <i>W. R. Q. Luke et al.</i> 3754 (MO)	EF456248; B: B; B
	<i>Strophanthus boirinii</i> Baill.	cult., Switzerland, Bot. Gard. Zurich, Dec. 2003, <i>M. E. Endress</i> s.n. (Z)	EF456192; B: B; B
	<i>Strophanthus caudatus</i> (L.) Kurz	cult., Thailand, Central Botanical Garden, Saraburi Prov., <i>D. J. Middleton</i> 245 (BKF)	EF456168; EF456577; EF456456; EF456290
	<i>Strophanthus preussii</i> Engl. & Pax	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 03-24 (BH)	EF456118; EF456539; EF456418; EF456252
	<i>Wrightia arborea</i> (Dennst.) Mabb.	Thailand, <i>D. J. Middleton</i> 240 (A)	EF456164; B: B; B
	<i>Wrightia coccinea</i> (Roxb. ex Hornem.) Sims	Thailand, <i>D. J. Middleton et al.</i> 897 (A)	EF456165; EF456527; EF456430; EF456287
	<i>Wrightia dubia</i> (Sims) Spreng.	Thailand, <i>D. J. Middleton et al.</i> 1399 (A)	EF456163; EF456526; EF456420; EF456257
	<i>Wrightia lanceolata</i> Kerr	Thailand, <i>D. J. Middleton et al.</i> 288 (A)	EF456169; EF456528; EF456433; EF456291

Appendix 1. Continued.

Subfamily, Tribe	Genus species Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer; <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
Apocynoideae, incertae sedis	<i>Wrightia religiosa</i> (Teijsm. & Binn.) Benth. ex Kurz	Thailand, <i>D. J. Middleton</i> 2033 (A)	EF456170; EF456530; EF456434; EF456292
	<i>Wrightia sirikitiae</i> D. J. Middleton & Santisuk	Thailand, <i>D. J. Middleton & Wongprasert</i> 579 (A)	EF456166; EF456531; EF456431; EF456288
	<i>Galactophora schomburgkiana</i> Woodson	Venezuela, <i>F. Michelangeli</i> 516 (PORT, VEN)	EF456178; EF456582; EF456406; EF456300
Asclepiadoideae, Asclepiadeae	<i>Asclepias syriaca</i> L.	U.S.A., New York, <i>T. Livshultz</i> 03-33 (BH, GH)	EF456111; B; B; B
Asclepiadoideae, Ceropegieae	<i>Vincetoxicum rossicum</i> (Kleop.) Barbar.	U.S.A., New York, <i>T. Livshultz</i> 03-31 (BH, GH)	EF456113; EF456651; EF456413; EF456381
Asclepiadoideae, Fockeae	<i>Heterostemma piperifolium</i> King & Gamble	Thailand, <i>D. J. Middleton</i> 194 (A)	EF456110; EF456610; EF456384; EF456380
	<i>Fockea edulis</i> K. Schum.	cult., U.S.A., Cornell University, 31 March, 1998, <i>T. Livshultz</i> s.n. (BH)	EF456115; EF456653; EF456405; EF456383
Asclepiadoideae, Marsdenieae	<i>Marsdenia glabra</i> Costantin	Thailand, <i>D. J. Middleton et al.</i> 1123 (A)	EF456114; EF456652; EF456414; EF456382
	<i>Telosma cordata</i> (Burm. f.) Merr.	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 01-33 (BH)	EF456112; B; B; B
Periplocoideae	<i>Cryptolepis sinensis</i> (Lour.) Merr.	Thailand, <i>D. J. Middleton et al.</i> 1016 (A)	EF456104; EF456644; EF456407; EF456374
	<i>Finlaysonia insularum</i> (King & Gamble) Venter	Thailand, <i>D. J. Middleton et al.</i> 1164 (A)	EF456105; EF456645; EF456408; EF456375
	<i>Gymnanthera oblonga</i> (Burm. f.) P. S. Green	Australia, <i>A. Ford</i> 2607 (BRI)	EF456106; EF456646; EF456409; EF456376
	<i>Petopentia natalensis</i> (Schltr.) Bullock	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 03-2 (BH)	EF456107; B; B; B
	<i>Phyllanthera grayi</i> (P. I. Forst.) Venter	Australia, <i>P. I. Forster</i> 24232 (BRI)	EF456103; B; B; B
Rauvolfioideae, Alyxieae	<i>Raphionacme flanaganii</i> Schltr.	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 03-23 (BH)	EF456108; EF456647; EF456410; EF456377
	<i>Zygostelma benthamii</i> Baill.	Thailand, <i>D. J. Middleton et al.</i> 849 (A)	EF456109; EF456648; EF456399; EF456378
	<i>Alyxia grandis</i> P. I. Forst.	Australia, <i>D. J. Middleton</i> 703 (A)	EF456089; EF456518; EF456393; EF456369
	<i>Alyxia oblongata</i> Domin	Australia, <i>D. J. Middleton</i> 702 (A)	EF456090; EF456519; EF456394; EF456370
	<i>Alyxia reinwardtii</i> Blume	Thailand, <i>D. J. Middleton</i> 172 (A)	EF456091; B; B; B
	<i>Alyxia spicata</i> R. Br.	Australia, <i>D. J. Middleton</i> 701 (A)	EF456092; EF456521; EF456395; EF456371
	<i>Chilocarpus costatus</i> Miq.	Thailand, <i>D. J. Middleton et al.</i> 370 (A)	EF456096; B; B; B
	<i>Chilocarpus rostratus</i> Markgr.	Malaysia, Sabah, <i>S. Davies et al.</i> 99272 (A)	EF456097; EF456523; EF456391; –
	<i>Condylocarpon isthmicum</i> (Vell.) A. DC.	Brazil, <i>L. S. Kinoshita et al.</i> 98-267 (UEC)	EF456087; B; B; B
	<i>Plectaneia thouarsii</i> Roem. & Schult.	Madagascar, <i>J. S. Miller et al.</i> 8769 (MO)	EF456102; EF456529; EF456402; EF456373
	<i>Pteralyxia kauaiensis</i> Caum	U.S.A., Hawaii, <i>D. Lorence</i> 7768 (PTBG, Z)	EF456098; EF456520; EF456470; –
Rauvolfioideae, Carisseae	<i>Carissa spinarum</i> L.	Thailand, <i>Newman et al.</i> 1131 (A)	EF456093; EF456591; EF456400; EF456372
Rauvolfioideae, Plumerieae	<i>Allamanda schottii</i> Pohl	cult., U.S.A., Cornell University, <i>T. Livshultz</i> 03-26 (BH)	EF456099; B; B; B
	<i>Cerbera manghas</i> L.	Thailand, <i>D. J. Middleton et al.</i> 2047 (A)	EF456094; B; B; B

Appendix 1. Continued.

Subfamily, Tribe	Genus species	Author	Voucher provenance, collector number (Herbarium acronym)	GenBank accession # <i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer; <i>rps16</i> intron, <i>rpl16</i> intron; <i>matK</i> and 3' <i>trnK</i> intron
Secamonoideae	<i>Cerbera manghas</i> L.		cult., Belgium, Nat. Bot. Gard. Belgium #77-0211, <i>F. Billiet S526</i>	EF456101; B*; B*; B*
	<i>Cerbera odollam</i> Gaertn.		Malaysia, Sabah, <i>Davies et al.</i> 99280 (A)	EF456095; EF456522; EF456392; EF456365
	<i>Himatanthus bracteatus</i> (A. DC.) Woodson		French Guiana, <i>S. Mori</i> 25799 (NY)	EF456161; EF456525; EF456396; EF456366
	<i>Plumeria cubensis</i> Urb.		cult., U.S.A., Hawaii, Nat. Trop. Bot. Gard. Kauai #860059003, Aug. 2003, <i>M. E. Endress s.n.</i> (PTBG)	EF456100; B; B; B
	<i>Theretia peruviana</i> K. Schum.		cult., U.S.A., Univ. Conn., <i>T. Lirshultz</i> 03-34 (GH)	EF456088; B; B; -
	<i>Secamone elliptica</i> R. Br.		Thailand, <i>D. J. Middleton et al.</i> 1162 (A)	EF456116; B; B; B
	<i>Toxocarpus villosus</i> (Blume) Decne.		Thailand, <i>D. J. Middleton et al.</i> 1341 (A)	EF456117; EF456649; EF456398; EF456379

APPENDIX 2. Morphological characters, coding, and discussion.

1. **Growth form:** 0 = self-supporting; 1 = dependent. Self-supporting encompasses herbs, shrubs, and trees that hold their branches without assistance from other structures. Dependent encompasses herbaceous or woody vines and lianas that either twine around or lean against other structures to support their branches. While both self-supporting and dependent are emergent properties of potentially non-homologous aspects of plant anatomy and physiology, this is the best possible coding without extensive anatomical and physiological investigation. Species that have been reported both as self-supporting and dependent or as somewhere in between, e.g., semi-twining in field notes of *Steyermark* 58220 for *Salpinctes kalmiifolius* Woodson, are coded as polymorphic for this character.

Most Wrightieae and Malouetieae are self-supporting, although some are dependent, e.g., *Alafia* Thouars, *Isonema* R. Br., *Farquharia* Stapf, many species of *Strophanthus* DC. Dependent growth forms predominate in the rest of the APSA clade, although many taxa occurring in more xeric, e.g., *Adenium* Roem. & Schult., or temperate, e.g., *Apocynum* L., *Asclepias* L., climates are self-supporting.

2. **Collecters on adaxial surface of leaf (petiole or lamina):** 0 = absent; 1 = present. Collecters on the base of the adaxial surface of the leaf lamina are characteristic for almost all species of Asclepiadoideae, some species of Secamonoideae, and most species of tribe Mesechiteae of subfamily Apocynoideae. The genera *Farquharia* (Malouetieae), *Isonema* and *Verium* L. (Wrightieae), *Baissea* A. DC., *Oncinotis* Benth., and *Motandra* A. DC. (Apocynaceae) have collecters primarily on the adaxial surface of the petiole, in some species extending to the junction of petiole and lamina and onto the base of the leaf lamina. Given the ambiguity in distinguishing the apex of the petiole and the base of the leaf lamina, collecters anywhere on the adaxial surface of the leaf, superaxillary collecters sensu Pichon (1949), are coded as homologous.

3. **Corolla aestivation:** 0 = sinistrorse; 1 = dextrorse; 2 = valvate. Dextrorse aestivation of corolla lobes in the floral bud is one of the diagnostic characters for the APSA clade, but sinistrorse aestivation occurs in *Wrightia* R. Br., *Stephanostema* K. Schum., *Pleioceras* Baill., *Parameria* Benth., and *Toxocarpus* Wight & Arn. Valvate aestivation is diagnostic for tribe Ceropegieae of Asclepiadoideae and occurs in some genera of Apocynoideae including *Urceola* Roxb. and *Parsonsia* R. Br.

4. **Fused basal tube:** 0 = absent; 1 = present. In Rauvolfioideae and Apocynoideae, the stamens are inserted atop a congenitally fused stamen–corolla tube (Nishino, 1982). In Periplocoideae, the stamens are typically inserted atop five, separate, more or less prominent lobes that often also bear a corona lobe at their apices, abaxial to the stamens. These lobes have been called the basal tube (Kunze, 1990), staminal feet (Endress & Bruyns, 2000), or basal corona lobes (Simões et al., 2007b). In Asclepiadoideae and some Secamonoideae and Periplocoideae, the anthers are borne atop a fused tube of tissue that arises as a solid ring and tightly encircles the gynoecium. In many Asclepiadoideae, corona lobes are also borne on this tube (termed the basal tube by Endress & Bruyns [2000]), abaxial to the anthers. Most workers have interpreted this tube in Asclepiadoideae as fused staminal filaments (Woodson, 1954; Sattler, 1973; Kunze, 1996), unfused or fused only at the very base in Secamonoideae (Safwat, 1962; Kunze, 1996), but Endress and Bruyns (2000) proposed that it is a specialized type of ring corona and that the anthers of

Asclepiadoideae and Secamonoideae are sessile at its apex. We have accepted this latter interpretation for this analysis. Because the topological relationship of the stamen–corolla tube, the staminal feet, and the basal tube to the stamens is the same (i.e., the stamens are inserted atop each of these), we here interpret the latter two as outgrowths of the stamen–corolla tube. We code all taxa except *Phyllanthera* Blume, Secamonoideae, and Asclepiadoideae as lacking a fused basal tube in order to contrast them with the latter three, which are coded as sharing the presence of a fused basal tube.

5. **Lignified anthers:** 0 = absent; 1 = present. Lignification of the anthers is diagnostic for the APSA clade but also characterizes most Tabernaemontaneae (Rauvolfioideae; Fallen, 1986; Simões et al., 2007b). It is absent in most Periplocoideae (Nilsson et al., 1993; Endress & Bruyns, 2000) and in *Holarrhena* R. Br. (Endress et al., 1990) and has been the basis for some workers associating these taxa with Rauvolfioideae rather than with the APSA clade (Schumann, 1895; Wanntorp, 1988).

6. **Retinacle:** 0 = agglutinated trichomes; 1 = cellular fusion. Union between the apex of the gynoeceium (the style-head) and the stamens, forming a gynostegium, is a synapomorphy for the APSA clade, present in all included taxa, although the attachment is very weak in some Malouetieae and Wrightieae, e.g., *Holarrhena*, *Nerium*. The region of the stamen by which it attaches to the style-head to form the gynostegium was termed the retinacle by Pichon (1948b) and used by him as the basis of his tribal classification (Table 2). More detailed studies using anatomical sections showed that retinacles are formed either by cellular fusion or by agglutination via trichomes (Nilsson et al., 1993; Kunze, 1996). Retinacles formed by cellular fusion have been found in all Periplocoideae, Secamonoideae, and Asclepiadoideae studied to date. Simões et al. (2004) also found formation of the retinacle via cellular fusion in some of the genera Pichon described as having a retinacle of a glabrous facet (*Allomarkgrafia* Woodson, *Mesechites* Müll. Arg., and *Mandevilla* Lindl.). Conversely, anatomical sectioning has shown that *Elytropus* Müll. Arg., *Baissea*, *Motandra*, and *Oncinotis* (described by Pichon [1948b] as having a glabrous facet retinacle) have retinacles formed by agglutination of trichomes (Nilsson et al., 1993; M. E. Endress, unpublished). Retinacle anatomy has yet to be studied in many genera of Apocynoideae, but we have chosen to include it because it is a potential synapomorphy for Periplocoideae, Secamonoideae, and Asclepiadoideae. We have coded cellular fusion as present in these three subfamilies as well as in *Allomarkgrafia*, *Mesechites*, and *Mandevilla*. *Forsteronia* G. F. W. Mey. is scored as polymorphic for this character following Simões et al. (2004). Genera described as having a glabrous facet retinacle by Pichon (1950a) but not yet studied in anatomical sections (*Parameria* Benth., *Ichnocarpus* R. Br., *Epigynum* Wight, and *Sindechites* Oliv.) are scored as unknown for this character. The rest of Apocynoideae are scored as having a retinacle formed by trichomes, and the outgroup genera are scored as inapplicable.

7. **Pollen transport adhesive:** 0 = a foamy or gel-like adhesive that may harden as the flower desiccates, forming amorphous translators; 1 = five translators each with a hardened corpusculum (Secamonoideae and Asclepiadoideae) or stalk (Periplocoideae) at anthesis, bifid in cross section, secreted in alternistaminal grooves on the style-head, with (most Asclepiadoideae) or without (most Secamonoideae) caudicles or with a basal adhesive pad viscidium and distal spoon (Periplocoideae). Presence of translators was the diagnostic character used by Brown (1810) to

separate Asclepiadaceae from Apocynaceae s. str. (Endress, 2004). However, amorphous translators, five discrete, hardened masses of adhesive positioned alternistaminally on the style-head, have been reported in *Apocynum* and *Forsteronia* (Nilsson et al., 1993). These observations were made on alcohol-preserved and dehydrated flowers. In fresh, unprocessed flowers of *Apocynum cannabinum* L. the pollen-transport adhesive has a gel-like consistency in flowers at anthesis and is hardened only in older, partially desiccated flowers. Which state of the pollen-transport adhesive functions in pollination and whether it functions as a translator (i.e., each mass of adhesive removed as a single unit with a load of pollen by the pollinator) remains unknown and can only be determined with pollination studies. To date, none of the pollination studies conducted on *Apocynum* have addressed this question (Waddington, 1976; Johnson et al., 1998; Lipow & Wyatt, 1999), and none have been conducted on *Forsteronia*. The restriction or not of adhesive secretion to five discontinuous alternistaminal zones on the style-head could be coded as a character, but we do not have sufficient information on its occurrence in Apocynoideae to score it at this time. Translators are coded as present only in Periplocoideae, Secamonoideae, and Asclepiadoideae, where removal and transport of translators by pollinators has been documented (Ollerton & Liede, 1997). There are many structural and developmental similarities among translators of Periplocoideae, Secamonoideae, and Asclepiadoideae (Safwat, 1962; Kunze, 1993; Endress, 2001). Omlor (1996) suggested that translators of Periplocoideae are not homologous with those of Secamonoideae because of differences in their histochemistry during development. It is not necessary, however, for structures to be identical at every level of organization in order to be treated as potential homologs in a cladistic analysis (Freudenstein et al., 2003). Differences in histochemistry among translators and other style-head secretions could be coded as additional characters, but we do not have sufficient data to do so at this time.

8. **Nectar secretion:** 0 = absent or not evident; 1 = on lobes around ovary, these sometimes fused into an annulus; 2 = in the alternistaminal sectors of the staminal feet or the fused basal tube. Most Apocynoideae have prominent lobed or annular nectaries surrounding the ovary. Exceptions are *Wrightia*, *Pleioceras*, *Stephanostema*, *Nerium*, *Adenium*, *Isonema*, *Alafia*, *Strophanthus*, *Farquharia*, and *Holarrhena*; these are coded as absent or not evident because we cannot rule out nectar secretion from some other structures at this point. In the Periplocoideae, nectar is secreted in five alternistaminal sectors on the margins of the staminal feet (Venter & Verhoeven, 2001). In Secamonoideae and Asclepiadoideae, it is secreted in the alternistaminal sectors of the fused basal tube (Omlor, 1996; Endress & Bruyns, 2000). Because we have chosen to interpret the staminal feet and the fused basal tube as outgrowths of the stamen–corolla tube (char. 4), we here code the presence of nectaries in alternistaminal sectors of the stamen–corolla tube as homologous for Secamonoideae, Periplocoideae, and Asclepiadoideae.

9. **Micropylar coma on seed:** 0 = absent; 1 = present. A micropylar coma is present in most taxa in the APSA clade, but absent in *Wrightia*, *Pleioceras*, *Stephanostema*, *Kibatalia* G. Don., *Funtumia* Stapf, and *Malouetia* A. DC. The existence of taxa with a micropylar coma only, the most frequent state in the APSA clade, with a chalazal coma only, with both micropylar and chalazal comas (*Adenium*, *Strophanthus*, *Isonema*, and *Farquharia*), and with no coma (*Malouetia*), indicates that presence or absence of a coma at one end of the seed is independent of presence or absence at

the other end of the seed and should be treated as two independent characters.

10. **Chalazal coma on seed:** 0 = absent; 1 = present. Chalazal comas are rare in the APSA clade, present only in *Wrightia*, *Pleioceras*, *Stephanostema*, *Kibatalia*, *Funtumia*, *Adenium*, *Strophanthus*, *Isonema*, and *Farquharia* (Van der Ploeg, 1983; Sennblad et al., 1998). All genera with chalazal comas have been classified in the tribe Wrightieae and/or Malouetieae in recent classifications.

11. **Pollen apertures:** 0 = absent; 1 = present. Most Rauvolfioideae and Apocynoideae have aperturate pollen with colporate or porate apertures, respectively. An exception is *Condylocarpon* Desf. (Rauvolfioideae, Alyxieae) with inaperturate pollen (Van der Ham et al., 2001; Endress et al., 2007). Periplocoideae have porate pollen; Secamonoideae and Asclepiadoideae have inaperturate pollen (Verhoeven & Venter, 2001; Verhoeven et al., 2003).

12. **Pollen unit at maturity:** 0 = monads; 1 = tetrads. Pollen in most Apocynaceae is dispersed as monads; tetrads are present in *Condylocarpon* (Van der Ham et al., 2001), *Apocynum* (Nilsson et al., 1993), Periplocoideae, Secamonoideae, and Asclepiadoideae. Pollinia of Asclepiadoideae other than *Fockea* Endl. have been described as composed of single pollen grains (Verhoeven et al., 2003); however, there is no free microspore stage in the development of these pollinia, rather the linear microspore tetrads are obscured as the grains enlarge and displace each other (Safwat, 1962; Dan Dicko-Zafimahova, 1980).

13. **If tetrads, dispersed as:** 0 = solitary tetrads; 1 = tetrads coherent into pollinia. A pollinium is the coherent mass of the entire pollen content of single sporangium. All Secamonoideae and Asclepiadoideae have pollen aggregated

into pollinia, as do seven genera of Periplocoideae (Verhoeven & Venter, 1998; Jonta & Judd, 2007); two of these, *Gymnanthera* R. Br. and *Finlaysonia* Wall., are sampled here. The presence of pollinia is here treated as a potential homology shared by these genera with Secamonoideae and Asclepiadoideae. Taxa that have pollen in monads (char. 12, 0) are scored as inapplicable for this character.

14. **Pollinial wall:** 0 = absent; 1 = present. A sporopollenin wall enclosing the entire pollinium is present in all Asclepiadoideae studied to date except *Fockea* (Verhoeven et al., 2003). Pollinia of Secamonoideae and Periplocoideae also lack a pollinial wall (Verhoeven & Venter, 2001). This character is scored only for taxa that have pollinia (char. 13, 1).

15. **Tetrads:** 0 = outer and inner walls not differentiated; 1 = outer and inner walls differentiated. In tetrads of *Condylocarpon* (Rauvolfioideae), Periplocoideae, Secamonoideae, and *Fockea* (Asclepiadoideae) the inner walls are differentiated from the outer walls by the presence of intine bridges (Van der Ham et al., 2001; Verhoeven & Venter, 2001). No such differentiation of outer and inner tetrad walls is present in *Apocynum* (Nilsson et al., 1993) and Asclepiadoideae other than *Fockea* (Verhoeven & Venter, 2001). This character is scored as inapplicable for taxa with pollen dispersed as monads (char. 12, 0).

16. **Number of sporangia:** 0 = 4; 1 = 2. Reduction of sporangia from four to two is apomorphic for Asclepiadoideae (Kunze, 1996). The presence of four sporangia and four pollinia is plesiomorphic in the Secamonoideae and has been used to interpret the subfamily as transitional between Periplocoideae and Asclepiadoideae (Safwat, 1962).

Appendix 3. Matrix of 16 morphological characters, arranged alphabetically by genus and species. An asterisk (*) indicates polymorphic for all states; a question mark (?), unknown; a dash (-), inapplicable.

Species	1	6	11	16
<i>Adenium obesum</i>	001010001110		---	0
<i>Aganosma cymosa</i>	101010011010		---	0
<i>Aganosma marginata</i>	101010011010		---	0
<i>Aganosma schlechteriana</i>	101010011010		---	0
<i>Aganosma wallichii</i>	101010011010		---	0
<i>Alafia barteri</i>	101010001010		---	0
<i>Alafia thouarsii</i>	101010001010		---	0
<i>Allamanda schottii</i>	00000-010010		---	0
<i>Allomarkgrafia brenesiana</i>	111011011010		---	0
<i>Allotoonia agglutinata</i>	101010011010		---	0
<i>Allotoonia woodsoniana</i>	101010011010		---	0
<i>Alyxia grandis</i>	00000-000010		---	0
<i>Alyxia oblongata</i>	00000-000010		---	0
<i>Alyxia reinwardtii</i>	10000-000010		---	0
<i>Alyxia spicata</i>	10000-000010		---	0
<i>Amalocalyx microlobus</i>	101010011010		---	0
<i>Angadenia berteroi</i>	*01010011010		---	0
<i>Angadenia sagraei</i>	101010011010		---	0
<i>Anodendron oblongifolium</i>	101010011010		---	0
<i>Anodendron paniculatum</i>	101010011010		---	0
<i>Apocynum androsaemifolium</i>	0010100110110		-00	

Appendix 3. Continued.				
Species	1	6	11	16
<i>Apocynum cannabinum</i>	0010100110110			00
<i>Artia balansae</i>	101010011010			---0
<i>Asclepias syriaca</i>	0111111210011101			
<i>Baiassea multiflora</i>	111010011010			---0
<i>Beaumontia grandiflora</i>	101010011010			---0
<i>Carissa spinarum</i>	00000-000010			---0
<i>Cerbera manghas</i>	00000-000010			---0
<i>Cerbera odollam</i>	00000-000010			---0
<i>Chilocarpus costatus</i>	10000-000010			---0
<i>Chilocarpus rostratus</i>	10000-000010			---0
<i>Chonemorpha fragrans</i>	101010011010			---0
<i>Cleghornia malaccensis</i>	101010011010			---0
<i>Condylocarpon isthmicum</i>	10000-0000010			-10
<i>Cryptolepis sinensis</i>	1010011210110			-10
<i>Cycladenia humilis</i>	001010011010			---0
<i>Echites turriger</i>	101010011010			---0
<i>Echites umbellatus</i>	101010011010			---0
<i>Elytropus chilensis</i>	101010011010			---0
<i>Epigynum auritum</i>	10101?011010			---0
<i>Epigynum cochinchinense</i>	10101?011010			---0
<i>Epigynum griffithianum</i>	10101?011010			---0
<i>Epigynum ridleyi</i>	10101?011010			---0
<i>Farquharia elliptica</i>	111010001110			---0
<i>Fernaldia pandurata</i>	101010011010			---0
<i>Finlaysonia insularum</i>	1010011210111010			
<i>Fockea edulis</i>	1111111210011011			
<i>Forsteronia acouci</i>	11101*011010			---0
<i>Forsteronia corymbosa</i>	10101?011010			---0
<i>Forsteronia guyanensis</i>	11101*011010			---0
<i>Forsteronia velloziana</i>	11101*011010			---0
<i>Funturia africana</i>	001010010110			---0
<i>Funturia elastica</i>	001010010110			---0
<i>Galactophora schomburgkiana</i>	*01010011010			---0
<i>Gymnanthera oblonga</i>	1010011210111010			
<i>Heterostemma piperifolium</i>	1111111210011101			
<i>Himatanthus bracteatus</i>	00000-000010			---0
<i>Holarrhena curtisii</i>	001000001010			---0
<i>Holarrhena pubescens</i>	001000001010			---0
<i>Ichnocarpus frutescens</i>	10101?011010			---0
<i>Ichnocarpus polyanthus</i>	101010011010			---0
<i>Ichnocarpus rhombifolius</i>	101010011010			---0
<i>Ichnocarpus serpyllifolius</i>	101010011010			---0
<i>Isonema smeathmannii</i>	111010001110			---0
<i>Kibatalia macrophylla</i>	001010010110			---0
<i>Laubertia contorta</i>	101010011010			---0
<i>Malouetia bequaertiana</i>	001010010010			---0
<i>Malouetia tamaquarina</i>	001010010010			---0
<i>Mandevilla boliviensis</i>	111011011010			---0
<i>Mandevilla longiflora</i>	011011011010			---0
<i>Marsdenia glabra</i>	1111111210011101			
<i>Mascarenhasia arborescens</i>	001010011010			---0
<i>Mascarenhasia lisianthiflora</i>	001010011010			---0
<i>Mesechites trifidus</i>	111011011010			---0
<i>Motandra guineensis</i>	111010011010			---0
<i>Neobraea bahamensis</i>	001010011010			---0

Appendix 3. Continued.

Species	1	6	11	16
<i>Neobracea ekmannii</i>	001010011010	---	0	
<i>Neobracea valenzuelana</i>	001010011010	---	0	
<i>Nerium oleander</i>	011010001010	---	0	
<i>Odontadenia lutea</i>	101010011010	---	0	
<i>Odontadenia perrottetti</i>	101010011010	---	0	
<i>Odontadenia puncticulosa</i>	101010011010	---	0	
<i>Oncinotis tenuiloba</i>	111010011010	---	0	
<i>Pachypodium baronii</i>	001010011010	---	0	
<i>Pachypodium lamerei</i>	001010011010	---	0	
<i>Papuechites aambe</i>	101010011010	---	0	
<i>Parameria laevigata</i>	10001?011010	---	0	
<i>Parsonsia crebriiflora</i>	102010011010	---	0	
<i>Parsonsia eucalyptophylla</i>	102010011010	---	0	
<i>Parsonsia ferruginea</i>	102010011010	---	0	
<i>Parsonsia flexuosa</i>	102010011010	---	0	
<i>Parsonsia lata</i>	101010011010	---	0	
<i>Parsonsia longiflora</i>	102010011010	---	0	
<i>Parsonsia oligantha</i>	101010011010	---	0	
<i>Peltastes isthmicus</i>	101010011010	---	0	
<i>Peltastes peltatus</i>	101010011010	---	0	
<i>Pentalinon luteum</i>	101010011010	---	0	
<i>Petopentia natalensis</i>	1010011210110	-10		
<i>Phyllanthera grayi</i>	1011011210110	-10		
<i>Plectancia thouarsii</i>	10000-000010	---	0	
<i>Pleioceras barteri</i>	*00010000110	---	0	
<i>Plumeria cubensis</i>	00000-000010	---	0	
<i>Pottsia laxiflora</i>	101010011010	---	0	
<i>Prestonia coalita</i>	101010011010	---	0	
<i>Prestonia lagoensis</i>	101010011010	---	0	
<i>Prestonia mexicana</i>	101010011010	---	0	
<i>Prestonia portobellensis</i>	101010011010	---	0	
<i>Prestonia quinquangularis</i>	101010011010	---	0	
<i>Prestonia riedelii</i>	101010011010	---	0	
<i>Prestonia robusta</i>	101010011010	---	0	
<i>Prestonia tomentosa</i>	101010011010	---	0	
<i>Pteralyxia kauaiensis</i>	00000-000010	---	0	
<i>Raphionacme flanaganii</i>	1010011210110	-10		
<i>Rhabdadenia biflora</i>	101010011010	---	0	
<i>Rhabdadenia macrostoma</i>	101010011010	---	0	
<i>Rhabdadenia pohlii</i>	*01010011010	---	0	
<i>Rhodocalyx rotundifolius</i>	001010011010	---	0	
<i>Salpinctes kalmiiifolius</i>	*01010011010	---	0	
<i>Secamone elliptica</i>	1011111210011010			
<i>Secondatia densiflora</i>	*01010011010	---	0	
<i>Sindechites chinensis</i>	10101?011010	---	0	
<i>Sindechites henryi</i>	10101?011010	---	0	
<i>Spirolobium cambodianum</i>	001010011010	---	0	
<i>Stephanostema stenocarpum</i>	000010000110	---	0	
<i>Stipecoma peltigera</i>	101010011010	---	0	
<i>Strophanthus boivinii</i>	001010001110	---	0	
<i>Strophanthus caudatus</i>	101010001110	---	0	
<i>Strophanthus preussii</i>	*01010001110	---	0	
<i>Telosma cordata</i>	1111111210011101			
<i>Temnadenia odorifera</i>	101010011010	---	0	
<i>Temnadenia violacea</i>	101010011010	---	0	

Appendix 3. Continued.

Species	1	6	11	16
<i>Thevetia peruviana</i>	0	0	0	0
<i>Toxocarpus peruviana</i>	1	1	1	1
<i>Trachelospermum asiaticum</i>	1	0	1	0
<i>Trachelospermum axillare</i>	1	0	1	0
<i>Trachelospermum difforme</i>	1	0	1	0
<i>Trachelospermum jasminoides</i>	1	0	1	0
<i>Urceola lucida</i>	1	0	1	0
<i>Urceola micrantha</i>	1	0	1	0
<i>Urceola minutiflora</i>	1	0	1	0
<i>Urceola rosea</i>	1	0	1	0
<i>Vallaris solanacea</i>	1	0	1	0
<i>Vincetoxicum rossicum</i>	1	1	1	1
<i>Wrightia arborea</i>	0	0	0	0
<i>Wrightia coccinea</i>	0	0	0	0
<i>Wrightia dubia</i>	0	0	0	0
<i>Wrightia lanceolata</i>	0	0	0	0
<i>Wrightia religiosa</i>	0	0	0	0
<i>Wrightia sirikitiae</i>	0	0	0	0
<i>Zygostelma benthamii</i>	1	0	1	1

APPENDIX 4. Resurrected genera. Taxonomic changes requiring new combinations will be published separately.

Amphineurion (A. DC.) Pichon, Bull. Soc. Bot. Fr. 95: 215. 1948. TYPE: *Amphineurion marginatum* (A. DC.) D. J. Middleton. 1 species.

Microchites Miq., Fl. Ind. Bat. 2: 456. 1856. TYPE: *Microchites polyantha* (Blume) Miq. 10 species.

Rhodocalyx Müll. Arg., in Martius, Fl. Bras. 6(1): 172. 1860. TYPE: *Rhodocalyx rotundifolius* Müll. Arg. 1 species.

Salpinctes Woodson, in Gleason, Bull. Torrey Bot. Club. 58: 453. 1931. TYPE: *Salpinctes kalmiiifolius* Woodson. 2 species.

Thyrsanthella (Baill.) Pichon, Bull. Mus. Hist. Nat. Paris. Sér. 2. 20: 192. 1948. TYPE: *Thyrsanthella difforme* (Walter) Pichon. 1 species.